

ASSIMILATE PARTITIONING IN AVOCADO, PERSEA AMERICANA MILL.

By

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Competition between developing reproductive structures and leaves for limiting carbohydrates may cause abscission of avocado fruitlets, because they form in a pseudoterminal position and develop concurrently with new, apical leaves. The assimilation, partitioning and translocation of recent radiolabeled photoassimilates were measured throughout fruit development to test this hypothesis. Sucrose and mannoheptulose were the major sugars in phloem exudates. During the summer months, only sucrose was radiolabeled. During the winter months, radiolabel was recovered in both sucrose and mannoheptulose.

Abscising and persisting fruit exhibited similar growth kinetics until 4-5 days before fruit separation when the growth rate of the abscising fruit decreased. Abscising fruit (puncture-induced) accumulated more radiolabel in the seed coat than did non-abscising controls. Restriction of radiolabel movement into the punctured fruit

was evident within 24 hours of puncturing and assimilate movement into the fruit ceased within 72 hours. Fruit separation occurred 24-48 hours later. Avocado flowers were photosynthetic; however, they contributed little photoassimilate to fruit growth. At the earliest stage of distal leaf development, assimilates from proximal source leaves were translocated in equal proportions acropetally to the inflorescence and developing distal leaves and basipetally toward the roots. As the inflorescence began to bear fruit, 5-times more label moved acropetally toward the fruit and expanding distal leaves than basipetally from the source leaves. In the final stage of distal leaf development fewer radiolabeled assimilates moved acropetally toward the fruit and mature distal leaves than basipetally. Fruit and distal leaves were equally effective in mobilizing recent photoassimilates.

The import rate of developing leaves peaked at 22% final midvein length (FMVL) and was negligible at 37% FMVL. Export from the leaves began at 20% FMVL and peaked at 60% FMVL. The distribution of assimilates from source leaves depended on sink phyllotaxy; fruit having the same orthostichy as the labeled source leaf received up to 95% of the radioactivity recovered from all of the fruit. Flushes of leaves on fruit-bearing branches fixed more radiolabel and translocated radiolabeled assimilates more rapidly than leaves on non-fruit-bearing branches on the same tree. Girdling branches decreased translocation from leaves on non-fruit-bearing branches by 50% and from leaves on fruit-bearing branches by 15%.

These results suggest that there was no apparent competition for recent photoassimilates among or between fruit and developing leaves.

CHAPTER 1 GENERAL BACKGROUND AND HISTORY

The avocado belongs to the Lauraceae family and is one of the few commercially important members of the genus Persea. Three races or ecological groups of avocado have been defined (15): Mexican, Guatemalan and West Indian. The Mexican race originated in the mountains of Mexico and Central America. The Guatemalan race is native to the highlands of Central America and the West Indian race is a native of the lowlands of Central America and northern South America. However, many of the commercial cultivars are hybrids of these races and the mixed genotypes have permitted a wider geographic distribution than the original races.

Avocado cultivars of the Mexican race are adapted to the cool temperatures of the tropical and subtropical areas with a Mediterranean-type climate. They are the most cold tolerant of the three races and can survive temperatures below -7.8°C (15). Some cultivars survive temperatures as low as -10°C (15). The leaves characteristically have an anise odor. The fruit has a thin smooth skin and is relatively small ranging in size from 75-300 g. The fruit matures earlier than or simultaneously with the West Indian cultivars.

The West Indian race cultivars are well adapted to lowland tropical conditions, i.e., high rainfall and high temperatures. However, this race is highly cold sensitive, being severely injured

below -2.8°C and killed by temperatures of -4.4°C (15). The fruit is larger than that of the Mexican race but smaller than avocados of the Guatemalan race. The peel is smooth, leathery and sometimes glossy.

The Guatemalan cultivars are intermediate between the other races with respect to their climatic adaptation. This race is prevalent in the Central American highlands (1500–1800 m) (15). Here it grows under cool temperatures but is likely to be killed by temperatures less than -6.1°C . The fruit weighs between 340 and 600 gr and it has a thick and brittle skin. The fruit matures later than either of the other two races.

The tree architecture of the genus *Persea* according to Rauh's model is determined by a monopodial trunk which grows rhythmically and so develops tiers of branches (195). The branches are morphogenetically identical to the trunk. The meristems are not equivalent, which leads to the differentiation between the trunk and the branches. However, branch development is closely correlated to the rhythmic growth of the axes. Branching is by syllepsis with the lateral axis developing during the phase of extension of the terminal bud on the current shoot. The branch tier then becomes more diffuse. The tree has a shallow root system and the leaves are arranged in spirals and come out in flushes.

The flowers are lateral and do not affect the growth of the shoot system. The inflorescence is consistently lateral or pseudoterminal. The flowers develop on the first part of the renewal shoot and their position as lateral appendages is soon evident as the distal leafy shoot elongates. The inflorescences appear by the thousands with each inflorescence bearing hundreds of flowers.

The flower-bud system is derived from terminal and subterminal buds on the previous season's growth. The buds are usually mixed and contain both vegetative and floral primordia (44,46,158,164,165). As the shoot elongates the lateral branches become simply or highly branched structures which bear single flowers forming a cymose inflorescence (46). The terminal portion of the main axis remains vegetative and continues to grow. Occasionally shoot tips terminate in a flower bud; hence, no further elongation occurs (158,165). In Florida inflorescence development begins in November (44,46,165) and continues over a period of several months.

The flowers when fully open are approximately 12 mm wide (158). They are borne on cymose panicles and are hypogynous, trimerous and perfect in form. The calyx and corolla are called the perianth lobes. The calyx and corolla can be distinguished only by their position. The perianth is composed of two whorls (a three-parted calyx and a three-parted corolla) which are coherent and form a cup-like structure. Four whorls of stamens, each containing three members, are inserted into this cup structure. The fourth whorl is reduced to glandlike staminodes. The flower contains a single pistil which consists of one carpel and one ovule. The ovule is anatropus and has two integuments.

The vascular tissue (41,169) in the pedicel is arranged in a siphonostele. Cross sections indicate six areas of primary xylem. Toward the receptacle the six perianth traces depart from the stele and pass through the cortex. The gaps left by the shift of the perianth vascular traces disappear; however, the vascular tissue of the stele shrinks as it approaches the receptacle. The carpellary traces depart near the tip of the axis and enter the ovary just before the vascular

tissue fades away. The vascular supply to the ovary consists of a dorsal trace, two or more lateral traces and two ventral traces which are united as a single vascular bundle.

The ventral trace enters the funiculus of the ovule and runs down the opposite side of the ovule near the dorsal part (41). This bundle branches profusely providing the integument with an ample vascular system. These branches are evident in the seed coats of mature fruits. The two dorsal and two lateral carpellary traces extend up through the style. The dorsal trace extends into the stigma. The lateral traces disappear in the style.

The flower although perfect is dicogamous. The pistil matures before the pollen is shed. Each flower opens twice at a distinctive time (179). At the first opening the pistil is receptive but the pollen is not shed. At the second opening the pollen is shed but the pistil is no longer receptive. The first and second opening under normal conditions are separated by one night. The distinctive time of opening has led to the formation of two classes, i.e., class A type and class B type. Class A cultivars open for the first time in the morning and for the second time during the afternoon of the next day. Class B cultivars open first in the afternoon and then reopen the next morning. In both cases during the first opening the flower is functionally pistillate. Even with the presence of active pollinating agents only .1% of the flowers will produce mature fruit (148).

The avocado has three distinct growing periods (44,133,168,169,171). The first period begins at the time of full bloom. The fruit enlarges rapidly and the integuments of the seed

develop, but the embryo remains small. Cell division during this period is very rapid and is marked by a homogeneous cell population size. During the second stage of growth the embryo develops, but the fruit overall does not enlarge. The third phase begins when the embryo stops growing and the fruit continues to grow. When the fruit has reached approximately 1/2 of its final size, the cells have also reached their final dimensions (average cell size of pericarp cells is 50-60 microns). Unlike most other fruits whose cells simply enlarge during phase three, the avocado cells continue to divide until harvest. The gross development of the avocado is represented by a sigmoidal curve (167,169). However, when examined more closely, the growth of the avocado has a diurnal cycle (171) in which the fruit contracts during the day and swells at night getting progressively larger each day.

The avocado fruit is a berry with one carpel and contains a single seed (15). The pericarp consists of three layers: the exocarp (skin and rind), fleshy mesocarp (edible portion of the fruit), the endocarp (thin inner layer next to the outer seed coat). The exocarp is the outer layer of the fruit and is covered by a wax-like cuticle. The exocarp is composed of one layer of epidermis, 1-3 layers of hypodermal cells, several layers of parenchyma cells and a layer of sclerenchyma cells which line the inner surface of the peel. The mesocarp is made up of uniform isodiametric parenchyma cells. Oil cells or idioblasts are spread throughout the mesocarp and can be identified by their large size and the lignified walls. The mesocarp is well vascularized in an

asymmetrical pattern. The endocarp consists of a few rows of parenchyma cells. These cells are smaller than those of the mesocarp and they adhere to the seed coat.

Research in Avocado

Avocado research in the last 15 years has focused primarily on three different areas. These are the fruit's nutritional value for human consumption, post harvest and climacteric behavior and morphology during development and abscission.

The fruit has been a major food source for the people of Central America for several thousand years, and with the advent of cold storage the fruit's popularity has spread worldwide. The less tropical avocados contain about 124 calories per 100 grams of fresh weight (84). The fruit contains between 4 and 10 percent protein (84). Avocado fruit provides approximately 666 mg/100 gr fresh weight of the essential amino acids (84). This is six times the amount provided by apples and three times the amount provided by either peaches or oranges (84). The avocado is exceptionally rich in minerals, oils and a number of vitamins (84).

The avocado is unique in several ways. In many fruits the final stages of growth result from cell expansion; however, in avocado, cells continue to undergo mitosis throughout the fruits sojourn on the tree (169). Furthermore, the avocado fruit does not ripen while it is still attached to the tree (2). Once removed from the tree, the fruit if sufficiently developed will undergo a climacteric rise in respiration and will ripen or soften. The length of time between picking and

ripening depends upon both the stage of development of the fruit and the cultivar. If the fruit is not picked, it may be 'stored' on the tree from anywhere between three months and one year.

The avocado is also unique in that it undergoes continuous drop throughout its development. In other fruits such as apple and orange a period of abscission follows anthesis (June drop). Fruit remaining on the tree through this period continues on to maturity. In avocado, depending on the cultivar, approximately 85% of the fruit set will abscise during the season (148). However, they do not abscise during a limited period but may abscise anytime from immediately post anthesis until harvest. The fruitlets that abscise are characterized by a discoloration of the seed coat and mesocarp (1,10,18,47), by an increase in ethylene production (1), an increase in respiration (14), and an increase in peroxidase isoenzymes and peroxidase activity (8,9,114,115,116,174,175).

As avocado fruitlets mature their respiration decreases (4). Ethylene production also decreases. Ethylene can no longer be detected in the fruitlet once it reaches approximately 10% of its final mass. The initial burst of ethylene is thought to be stimulated by pollination (4) or result from the ongoing abscission process (46).

An increase in respiration and ethylene production occurs when the fruit is removed from the tree (1,46). Fruitlets at all developmental stages show a similar climacteric pattern when harvested (205). However, the postharvest preclimacteric period shortens as the fruit develops. Seed respiration is high in young fruits and decreases with fruit development (205).

The role of ethylene in the ripening of fruit has not been determined. Ethylene does not induce its own synthesis in immature fruitlets but it does induce its own synthesis in mature fruit (55,56). In fruit harvested during the early season, ethylene causes a temporary increase in respiration rates but does not trigger the climacteric pattern (55,56). Preclimacteric fruits contain less than .1 nmol/g of 1-amino-cyclopropane-1-carboxylic acid (ACC) (100). This immediate precursor of ethylene increases to 45 nmol/gr of tissue during the climacteric peak which in turn causes an increase in ethylene production. As ethylene is evolved the ACC content again drops. As the fruit becomes overripe ACC accumulates; however, ethylene is no longer generated. Ethylene has no effect on attached fruit (70). Freshly harvested fruit do not respond to ethylene for a least 24 hours suggesting that attached fruit are affected by some endogenous factor which inhibits ethylene stimulated ripening (17).

Blumenfeld and Gazit (17) described an endogenous growth regulator found in avocado fruit tissue. This agent inhibits indolacetic acid (IAA) and cytokinin-mediated activities. Avocado mesocarp also synthesizes a large quantity of ABA during development (135). The greatest synthesis occurs after the seed coat has broken its vascular connection with the mesocarp. The large increase in free ABA occurs two days after the peak of ethylene evolution. Although ABA may be involved in the ripening process, it is not responsible for ethylene evolution (17).

Various biochemical processes have been examined during the climacteric cycle. Lance et al. (125) reported that the increase in the respiratory activity of the preclimacteric fruit was probably due

to the increased availability of cofactors and not due to an increase in the number of mitochondria or an increase in ATPase activity. The level of the enzyme pectinmethylesterase remains constant through the preclimacteric but the enzyme's activity decreases with initiation of the respiratory climacteric (9). Conversely, the activity of the enzyme which depolymerises pectin, polygalacturinase, increases during the climacteric and post-climacteric periods (8). Cellulase activity in avocado also increases during the preclimacteric stage (8). Exo- and endo-cellulase activities are stimulated by ethylene (105,157).

Climacteric and post-climacteric avocado are characterized by browning tissues. The browning is caused by oxidases such as polyphenol oxidase (PPO), peroxidase (PO), and catalase (114,115,116 respectively). Polyphenol oxidase is actually a family of isozymes. In avocado there are at least six forms of the enzyme ranging in molecular weight from 14,000 to 400,000 daltons. These isozymes are good developmental markers since they appear or disappear at specific times during the climacteric cycle. Peroxidases also have multiple forms which appear at specific times in the climacteric cycle.

Abscission and Partitioning

The process of abscission is a widespread phenomenon in crop plants. There have been several hypotheses put forth to explain the abscission of plant organs. These hypotheses involve correlative plant behavior, inhibition and senescence, organ competition and plant homeostasis (3). Today, the relevant hypotheses concerning fruit abscission are inhibition and senescence, and organ competition (3).

Inhibition and senescence in relation to fruit abscission are actually two separate phenomena. Inhibition of abscission results from the interaction of hormones (3,24). The secretion of IAA, in particular, by leaves, fruits and other storage organs is proposed as one mechanism whereby abscission is inhibited. Abortion and senescence resulting in abscission occur when the organ in question physiologically degenerates. Degeneration is the result of improper development or a physiologically weak embryo (3).

The hypothesis that organ competition results in abscission stems from the concept that plants initiate and partially develop more leaves, buds, flowers and fruits than their stem and root systems can support. This results in competition between sinks for light and nutrients. This competition extends to vegetative and reproductive growth. Whether a fruitlet reaches maturity depends on its ability to remain a strong sink. If the fruitlet can not successfully compete, its reserves are remobilized (fruitlet now becomes a source) and eventually the fruitlet abscises.

Assimilate partitioning and translocation have been studied in several crops. Koch and Schrader (118) examined the allocation of ^{14}C -photosynthates in soybean from anthesis to harvest. They found that the amount of ^{14}C -labelled amino acids translocated from the source leaf to the pod varied with the developmental stage of the pod and these amino acids comprised 12-48% of the seeds' nitrogen. One-third of the ^{14}C -label present in the leaf blade was in the form of starch. During the late pod filling stage; however, this proportion

dropped dramatically. Sugars comprised 70-86% of the ^{14}C -label of the source leaf's soluble assimilates. The highest values occurred during late flowering and early pod filling stages.

Variation in the levels of assimilate accumulation had also been reported (23) between abscising and non-abscising soybean flowers and Mandarin orange (69). The sink intensity was very high in both abscising and non-abscising flowers before anthesis. During the first three days post-anthesis, the sink intensity decreased and remained low. In normally setting flowers the sink strength then began to increase while abscising flowers failed to recover their sink strength. Reproductive abscission in soybean is determined at a very early stage. Photosynthate accumulation correlates to reproductive abscission. However, whether variations in assimilate accumulation are a cause or an effect of abscission has yet to be determined.

In tomato (94), assimilate accumulation in the fruit was dependent on the developmental stage of the fruit and was independent of the rate of the CO_2 fixation in the source leaf. Translocation of photosynthate was regulated by sink demand. However, soybeans given supplemental light exhibited both reduced abscission and increased seed weight/node when compared to natural light controls. Shading of flowers and pods increased abscission and reduced seed weight. Shading of the flowers and pods resulted in a decrease in ^{14}C -assimilates from the source leaf. As the nodes were shaded the abscission percentage increased, even when supplemental lighting was provided. This suggested that flowers and pods play a role in regulating both their own assimilate accumulation and their own abscission via a perceived light stimulus.

Assimilates moving into the reproductive structures are generated in the vegetative portions of the plant. Recently fixed photosynthetic assimilates are preferentially translocated to the sink; however, remobilization of storage compounds may also occur. In soybean (29) girdling did not affect the ^{14}C -assimilate accumulation in pod walls or seeds; however, it did decrease the starch levels of the subtending leaf. Although sink demand remained constant, the source of readily available assimilate decreased when the plant was girdled. The plant compensated for this change by altering the partitioning of fixed carbon. The developing embryo may exert a direct effect on photosynthetic efficiency. The net CO_2 uptake in Capsicum annum was reduced by 30% when the fruits were removed. The stems of defruited plants exhibited pronounced changes in their soluble sugar and polysaccharide content. The decrease in photosynthate accumulation reflected the decrease in sink demand. The loss of photosynthetic capacity was due to the loss of both soluble fraction one proteins and RuBP carboxylase (82).

The ability of a plant to utilize stored reserves and the decrease in these reserves after a heavy fruit load have been used to explain alternate bearing (75,76,162). A heavy fruit load decreases the amount of stored reserves in the plant and decreases the plant's ability to produce fruit the next season. Olive is a notorious alternate bearing crop. Stutte and Martin (181) have shown that the carbohydrate reserves in olive have no affect on alternate bearing. They used CO_2 enrichment techniques to increase the starch content tenfold over the controls yet they saw no differences in the flowering pattern of the bearing and nonbearing trees.

Plant growth substances have been credited as the driving force of sink demand. Exogenous application of individual or multiple growth regulators to sinks increased the demand for assimilates (141). Growth regulators, therefore, directly affect translocation by stimulating the sink's synthetic activities which require assimilates. Gibberellic acid (GA) and its synthetic analogs decreased styler abscission, decreased the early drop of fruit and increased the early size of fruit (3). Gibberellic acid is thought to act by increasing auxin production. Auxin prevented abscission when applied to the sink distal to the abscission zone (3,31,131,154,182).

The role of the fruit as a source of hormones (3,131,202) and nutrients has complicated the early models of source-sink relationships. Unlike source to sink translocation which occurs primarily in the phloem, export from the sink to the source is via the xylem (14). Since mass flow in the xylem is nonselective, any exported compound may be translocated to all the vegetative parts of the plant. Removing soybean pods from the plant decreases dry matter accumulation and reduces the nitrogen content of the whole plant (37). Depodded plants also exhibited delayed leaf senescence, suggesting that the pods alter the partitioning of plant constituents and affect the initiation of senescence. In Phaseolus vulgaris (182) the older fruits at the base of the raceme aborted less frequently than the younger fruits. When the older fruits were removed, the abortion rate of the younger fruits decreased as did the ABA content of the fruits. This suggested that the older fruits have a direct regulatory effect on the ABA content and abscission of younger fruit.

CHAPTER 2
THE TRANSLOCATED SUGARS IN PERSEA AMERICANA MILL.

Introduction

Phloem exudates of some members of the Lauraceae family have been shown to contain several sugars. These include sucrose, raffinose, stachyose, verbascose and myoinositol (211). The major component of these transported sugars is sucrose. Zimmerman and Ziegler (211) identified sucrose as the only sugar translocated in the phloem of avocado, a member of the Lauraceae. Recent unpublished results, however, have led Blumenfeld (BARD project #I-414-81 Final Report) to suggest that the C7 sugars, perseitol and mannoheptulose are the major translocated sugars.

The occurrence of mannoheptulose in leaves and fruit is rare although not unique to avocado (30,203,205,210,211). Mannoheptulose was first identified in avocado fruit by LaForge (123). During avocado leaf photosynthesis labeling of sucrose is rapid and linear (11,12) while mannoheptulose is labeled at a linear rate one-third that of sucrose. Mannoheptulose appears to be a normal product of photosynthesis (11,12,146) generated from mannoheptulose phosphate by a phosphatase. Nordal and Benson (146) isolated mannoheptulose and mannoheptulose monophosphate from the leaves of avocado and suggested that a slow enzymatic exchange occurred between free and phosphorylated mannoheptulose.

Historically, research on the identity and quantity of substances translocated in the phloem has been retarded by difficulty collecting unadulterated phloem samples. Hartig (90) developed a bark incision technique which involved cutting through the phloem members and collecting the exuded sap in capillaries. Although this technique is commonly used, several factors may affect the integrity of the sample. These include loss of exudate to the xylem (206,209,210), alteration source loading (52), loss of phloem function (28,173), dilution and contamination of phloem exudate by the contents of damaged cells and the pressure release of non-phloem constituents (37,129,206). An alternate technique uses feeding aphids to penetrate individual sieve tube members (61,62,136,210). This technique is limited by the restricted feeding locations of aphids and by the limited host range of aphids. Avocado does not have any known aphid pest. Fortunately, under well-watered conditions, avocado petioles exude a sizable droplet of phloem contents when severed from the plant with little contamination from exposed cortical cells (personal observation). This novel characteristic allowed us to examine phloem exudates in individual leaves over time as related to sugar constituents.

Persea americana Mill. is comprised of three races or ecological groups: Mexican, Guatemalan and West Indian. These races vary in their cold tolerance (15) and fruit characteristics such as size, oil content, and skin texture (15,128,170). The possible discrepancy in the identity of the translocated sugars noted above may reflect inherent differences in races. This study was undertaken to identify the primary and

secondary translocated sugars in representatives of all three races of avocado as part of a larger effort to understand the partitioning of recent photoassimilates in avocado.

Materials and Methods

Leaf Extracts

The trees used in this study were three-year-old, containerized trees of Persea americana Mill. cv. 'Peterson' grafted onto 'Walden' root stock as well as 10-year-old 'Peterson' and 'Booth 8' orchard trees growing on 'Walden' root stock. Individual leaves on the most recent flush were sealed in 2-liter plastic containers and exposed to 2.25 uCi of $^{14}\text{CO}_2$ generated from $\text{NaH}^{14}\text{CO}_3$ (Amersham, specific activity of 54 mCi/mmol) using equimolar amounts of 5% lactic acid. The leaves were labeled for 15 minutes between 0900 and 1100 hr on cloudless days. The experiment was replicated for each of two leaves on six occasions during May.

Leaves were harvested immediately following the labeling period (0 hr), at 1 and 4 hours. They were excised, quickly separated into laminate and major vein portions, cut into small pieces and dropped into separate flasks containing approximately 100 ml boiling 85% ETOH according to Housely et al. (102). The leaves/veins were boiled for 15 minutes and filtered. The residue was further extracted with similar volumes of 50% ETOH and water. The sample extracts were pooled, rotoevaporated to dryness and reconstituted with 20 ml H_2O . Sugar content of the extracts was quantitated by the phenol-sulfuric acid method of Dubois et al. (53) to calculate the relative sucrose content and specific activity of the radiolabeled products. Radioactivity was

determined by scintillation spectroscopy of 1 ml samples. Some samples were spiked with sucrose for treatment with invertase and for comparison to unspiked samples.

Invertase (Sigma) was added to some extracts and control sugar samples, after acidification to pH 6 with 1N HCl, to reach a final concentration of 1 mg/ml. The samples were incubated for 30 minutes at 4°C. The pH of the samples was then adjusted to 7.5, by the addition of 30% NaOH, to stabilize the fructose content. Thirty microliters were spotted on thin layer plates and developed as described below.

Invertase was used to more firmly identify the spot which comigrated with sucrose.

Phloem Exudates

The trees used in this study were mature, orchard trees growing in Rockdale soil at the Tropical Research and Education Center in Homestead, FL. The most recent growth flushes, each containing approximately 10 leaves, were enclosed in 3 gallon plastic bags and labeled with 20 uCi of $^{14}\text{CO}_2$ for 1 hour. Two branches of each cultivar were labeled on three different occasions during the late summer. Representative cultivars of each race and racial hybrids were chosen, they included 'Peterson' and 'Fuchs' (West Indian), 'Winter Mexican' and 'Mexicola' (Mexican), 'Itzamana' (Guatemalan), 'Booth 8' (Guatemalan X West Indian), and 'Fuerte' (Mexican X Guatemalan). Flushes were harvested at 4 and 24 hours after labeling. Each branch was cut from the tree and the cut end placed in a beaker of water before immediate transfer to the laboratory. Leaves were excised at the base of the petioles and the exudate collected with 5 ul capillary pipettes. The exudate (3 to 5 ul) was spotted directly onto TLC plates.

Thin Layer Chromatography

Silica FG plates were pre-activated by heating at 110°C for 1 hour. The cooled plates were spotted with aliquots of control sugars (glucose, fructose, sucrose, mannoheptulose, perseitol, galactose, sorbose, ribulose, sorbitol), laminate and vein extracts, invertase treated sugars and extracts and phloem exudates. The plates were air-dried and enclosed in a tank with a developing solution consisting of either chloroform:acetic acid:water (3:3.5:0.5) or acetone:water (9:1). The plates were removed when the solvent front had moved at least 10 cm. They were air dried for 30 min, then sprayed with aniline diphenylamine in acidified acetone (Sigma) and heated for 30 minutes at 80°C to enhance color development. Aniline diphenylamine is a differential spray reagent for the identification of non-reducing carbohydrates. Pentoses appear as red spots. Methylated aldopentoses appear cherry red. Aldohexoses appear brown. Hexauronic acid and methylated aldohexoses appear brownish red. Fully methylated aldohexoses appear maroon. Additionally, some plates were sprayed with concentrated H_2SO_4 and heated at 80°C for 30 min to detect any nonstaining organic components.

One-half of each plate was sprayed with the color developer. The unstained half of the plate with identical samples was scraped for scintillation counting. The R_f of radioactivity was determined by scraping 1 cm squares extending from below the origin to the solvent front of the TLC plate. The R_f and color of radioactive and stained spots of the extracts were compared to the stained standards for identification of the putative sugars in the extract.

Temperature Studies

The most recent flushes of leaves evident during Nov., Dec. and Jan. were cut from the tree and transported into the laboratory. Three leaves of approximately the same age were cut from the branch and each suspended in a 2 liter beaker. The beakers were half filled with ice, hot water (37°C) or water at ambient temperature (27°C). A vial containing 3 mL of 5% lactic acid was taped to the inside of the beaker. The beakers were covered with Saran wrap and allowed to equilibrate for 30 min, 60cm below a 400 watt lamp. Leaf temperatures were measured before and after the labeling period. After the equilibration period, 10 uCi of $\text{NaH}^{14}\text{CO}_3$ was added to the lactic acid. One hour later, the leaf temperature was measured again and the Saran wrap was removed. The leaves were minced and boiled in 85% ETOH as described earlier. The alcoholic extracts were used to spot TLC plates. The experiment was repeated three times.

Sugar Separation

Preparative TLC plates were spotted with control sugars and streaked with 100 ul of leaf extract and then developed in a chloroform:acetic acid:water solvent. The control strips were sprayed with aniline diphenylamine in acidified acetone. The areas of the unstained plate corresponding to the R_f of sucrose and mannoheptulose were scraped. The silica gel scrapings of each sugar were each extracted with 500 ul of water. The suspension was then centrifuged to sediment the silica gel. The sugar content of the supernatant was quantitated using the phenol-sulfuric acid method of Dubois et al. (53). Radioactivity in an aliquot of the supernatant was measured by liquid scintillation counting.

Results and Discussion

The goal of the present research was to identify the translocated sugar containing recently photoassimilated $^{14}\text{CO}_2$ and to address the discrepancy between previously reported results. Earlier workers reported either sucrose as the only translocated sugar (211) or suggested both sucrose and mannoheptulose (11,12,146) are formed during photosynthesis and both play a role in translocation. We observed only 2 major sugars, sucrose and mannoheptulose, present in ethanol extracts (Table 2-1), and only sucrose contained the radiolabel during the summer months (Fig. 2-1).

The phloem exudates from the petiole incisions of the tested cultivars contained both mannoheptulose and sucrose. These sugars were present in the phloem in similar amounts as indicated by their almost identical spot sizes on thin layer chromatography plates and as quantitated by the phenol sulfuric acid method after separation on preparative TLC plates. However, sucrose was the only labeled compound in the phloem exudates at 4 and 24 hours (Fig. 2-2). This was true for all races of avocado. These results indicate that sucrose was the sugar responsible for the movement from the leaves of recently photoassimilated carbon and remobilized leaf starch reserves.

Chromatograms of both laminate and vein extracts produced two spots (Table 2-1). One spot had the same mobility as sucrose and comigrated with sucrose in spiked samples. When treated with invertase this spot disappeared and two other spots appeared having the same mobility as fructose and glucose. The second spot was not affected by invertase (Table 2-1). It had the same mobility and staining characteristics as mannoheptulose. This sugar was never associated with the radiolabel during the summer months.

The profile of the radiolabel distribution at zero hour for the laminate extract is shown in Fig. 2-1. The majority of disintegrations per min (dpm) were found at the sucrose spot. Nonstaining metabolic intermediates account for the remainder of the extracted label. No radioactivity was present in the vein extract at this time point. At 1 hour, however, all of the ethanol extractable radioactivity was associated with the sucrose spot and radiolabel was detected in the vein extracts for the first time (Table 2-2). The radiolabel in the vein extracts was associated with the sucrose spot. During the 4 hour course of this experiment the proportion of sucrose in the laminate and vein extracts remained the same as did the proportion of radiolabel after 1 hour, indicating that the system was not disrupted by the labeling process and that the sample leaves were physiologically comparable.

During the winter months (Nov. to Jan.) recently fixed $^{14}\text{CO}_2$ was incorporated into both sucrose and mannoheptulose. The incorporation of label into either the sucrose or mannoheptulose pool was dependent on temperature (Fig. 2-3). At low temperatures (22°C) more label was incorporated into sucrose than into mannoheptulose (Fig. 2-3); more sucrose than mannoheptulose was also produced under these conditions (Fig. 2-4). These results were similar to those reported by Bean et al. (12) at a similar temperature. At the ambient temperature for that time of year (27°C), similar amounts of radiolabeled $^{14}\text{CO}_2$ were incorporated into sucrose and mannoheptulose, and they were being produced at similar rates. At a higher temperature (37°C), the amount of label recovered in sucrose decreased although sucrose was still preferentially labeled over mannoheptulose (Fig. 2-4).

Persea americana Mill. consists of three ecologically distinct races: Mexican, Guatemalan and West Indian. We did not observe any differences in the sugar compliment of the phloem of avocado varieties representative of the three races. In fact, all of the varieties tested had significant amounts of mannoheptulose and sucrose in their phloem and leaves. Race did not play an important role in the determination of the translocated sugar.

Seasonal changes did influence the partitioning of recently fixed label into the sugar pools. Although the reasons for this are not clear, several avenues of investigation are available. The winter months in Florida are cool and dry, suggesting mannoheptulose may act as an osmoticant. Although the experiment was conducted under water saturating conditions, the leaves used in the experiments were removed from field grown trees which had acclimated to the current winter conditions. However, at the experimentally induced lower temperature, sucrose, not mannoheptulose, appeared to be preferentially formed suggesting that mannoheptulose was not formed as a protection from frost. Flower initiation also occurs during the winter months. The production of mannoheptulose during the period of flower initiation is suggestive and deserves further examination.

In conclusion, avocado exhibited seasonal differences in the partitioning of recently photoassimilated carbon into its sugar pools. During the summer months, recently fixed carbon is recovered only in sucrose although mannoheptulose was present. During the winter months sucrose and mannoheptulose were both labeled in a temperature dependent manner.

Table 2-1. Thin layer chromatography of control sugars and leaf/vein extracts and phloem exudates.

	Chloroform: Acetic acid : Water		Acetone:Water
	Rf	color*	Rf
Leaf	.252	brown	.492
	.333	orange	.79
Vein	.249	brown	.492
	.333	orange	.79
Phloem exudate	.249	brown	
	.333	orange	

Invertase treatments			
Sucrose	.361	blue-grey	.516
	.385	orange	.543
Leaf	.333	orange	.79
	.361	blue-grey	.516
Vein	.385	orange	.543
	.333	orange	.79
	.361	blue-grey	.516
	.385	orange	.543

Sucrose	.249	brown	.492
Mannoheptulose	.333	orange	.79
Glucose	.361	blue-grey	.516
Fructose	.385	orange	.543
Invertase	----	-----	

Phloem exudates were obtained with 5 μ l capillary pipettes from cross-sectional cuts through the leaf petiole. Leaves were separated into laminate and major vein portions and dropped into boiling 85% EtOH. The leaves and veins were boiled for 15 and then filtered. The residue was washed with 1 volume of 50% EtOH followed by 1 volume of water. The extract was rotoevaporated and then spotted on pre-activated TLC plates. Samples were separated into vials for control and invertase treatments. Acidified extracts were treated with invertase (final concentration 1 mg/ml) and incubated at 4°C for one hour. The pH of the sample was then increased to 7.5 and then spotted on pre-activated TLC plates. The plates were developed in either a chloroform:acetic acid:water (3:3.5:0.5) or an acetone:water (9:1) solvent system. When the solvent front had moved at least 10 cm the plates were removed and air dried for 30 min. The plates were sprayed with analine diphenylamine in acidified acetone and heated at 80°C for 30 min. Analine diphenylamine in acidified acetone is a differential spray for the detection of non-reducing sugars.

Table 2-2. Pulse-Chase of Ethanol-Extracted Radiolabeled Sugars in Avocado

Tissue	Time	Extracted Sugars (mg)	dpm/mg [*]	% Leaf Sugars	% dpm ^{**}
Lamina	0	20.64	4.25	69.60	41.00
Vein		9.45	0.00	31.40	0.00
Lamina	1	19.37	10.65	69.00	79.31
Vein		8.68	2.78	30.90	20.69
Lamina	4	28.50	7.18	68.20	78.04
Vein		13.26	2.02	31.80	21.96

Leaves were labeled, harvested and prepared as described in the Materials and Methods. Sugars were quantified by the phenol-sulfuric acid method of Dubois et al.(53). Sugars specific activity is recorded as dpm/mg sugar. The distribution of sugars between the lamina and vein portions of the blade is indicated by the percent leaf sugars. The percent dpm refers to the amount of radiolabel associated with the sucrose spot on TLC plates for each leaf blade (lamina vein).

* Associated with sugars

** Associated with the sucrose spot

Figure 2-1. Profile of Radiolabel Distribution in Leaf Extracts at 0, 1 and 4 Hours after Labeling. Leaves were fed $^{14}\text{CO}_2$ and harvested immediately, or at 1 or 4 hours after labeling and then separated into lamina and major vein fractions and dropped into boiling 85% EtOH. The leaves and veins were boiled, filtered and the residue was washed with 1 volume each of 50% EtOH and water. The extract was rotoevaporated and then spotted on pre-activated silica FG plates which were developed in a chloroform:acetic acid:water (3:3.5:0.5) solvent. The plates were air dried after the solvent front had moved at least 10 cm. One cm squares were then scraped into scintillation vials and counted. The peak of radioactivity which comigrates with sucrose is marked by an *. Location of mannoheptulose is indicated by a +.

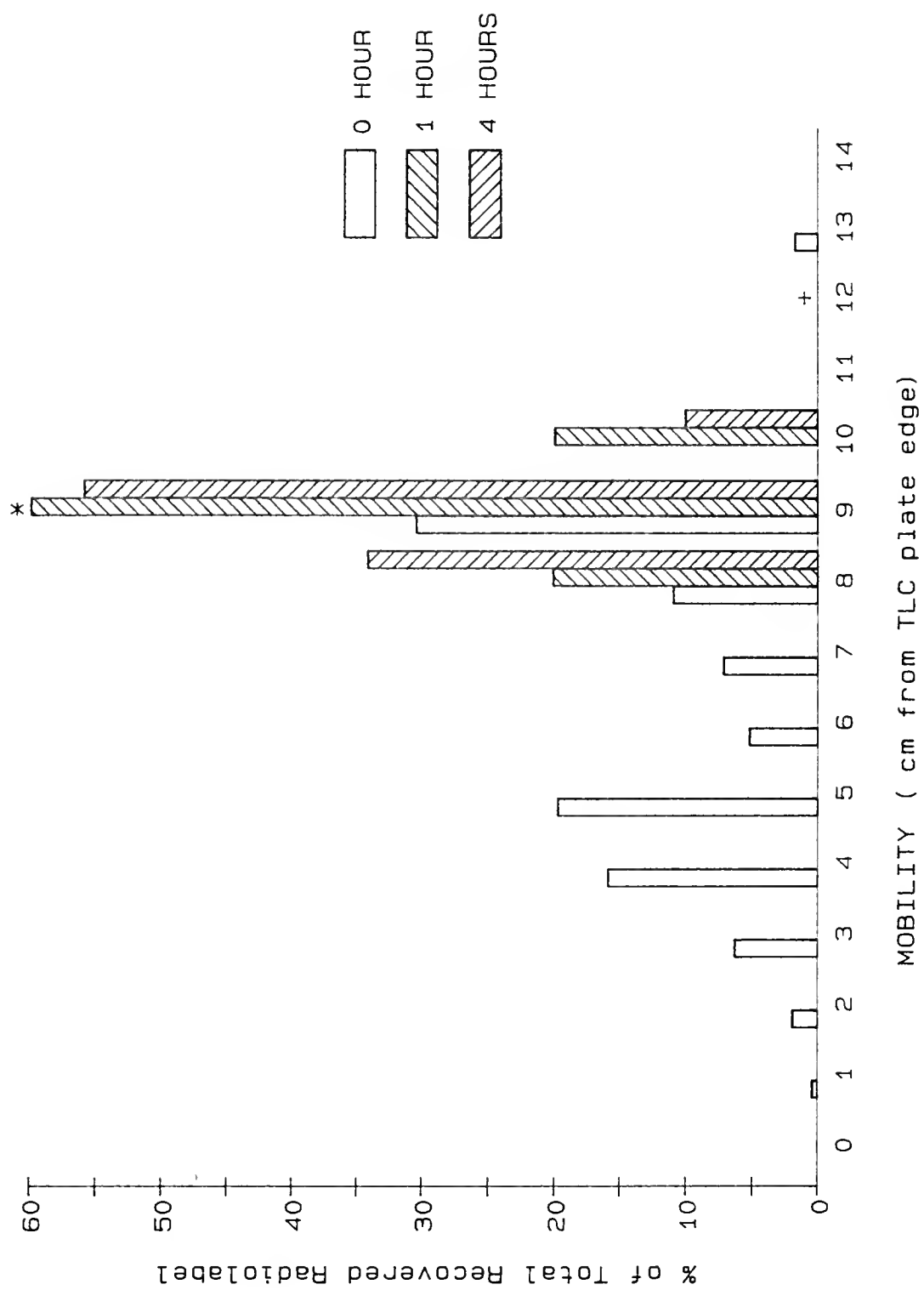


Figure 2-2. Profile of radiolabel distribution from phloem exudates. Leaves were fed $^{14}\text{CO}_2$ and harvested immediately, or at 4 or 24 hours after labeling. Phloem exudates were collected in capillary pipettes and applied in 0.25 ul quantities to pre-activated silica FG chromatography plates. Similar patterns were seen with all races of avocado. The peak of radioactivity which comigrates with sucrose is marked by an *. The location of mannoheptulose is indicated by a +.

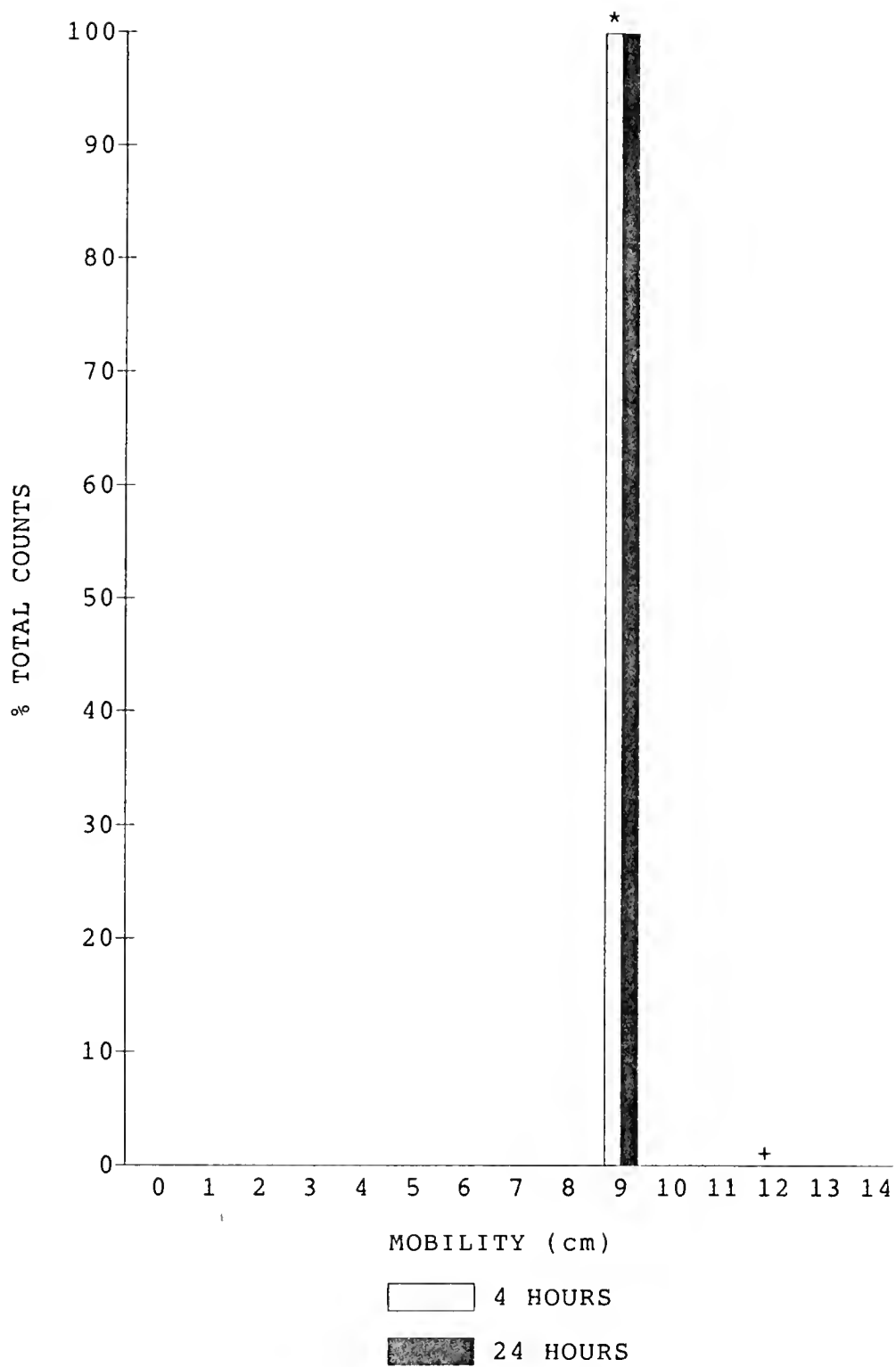


Figure 2-3. Distribution of ^{14}C in Sugars of Avocado Leaves at Various Temperatures. Leaves were incubated at three temperatures and fed $^{14}\text{CO}_2$. At the end of the incubation period the leaves were macerated and boiled in 85% EtOH. Leaf extracts were spotted on TLC plates. The plates were developed in a chloroform:acetic acid:water solution. The plates were removed from the developing solution and scraped for sugar extraction and scintillation spectroscopy. The silica gel scrapings were extracted with 500 μl of water. Sugar content was measured by the phenol-sulfuric acid method. Radioactivity was measured by scintillation spectroscopy. The histogram bars indicate the relative percentage of total leaf dpm recovered from the silica gel corresponding to the location of the sugar.

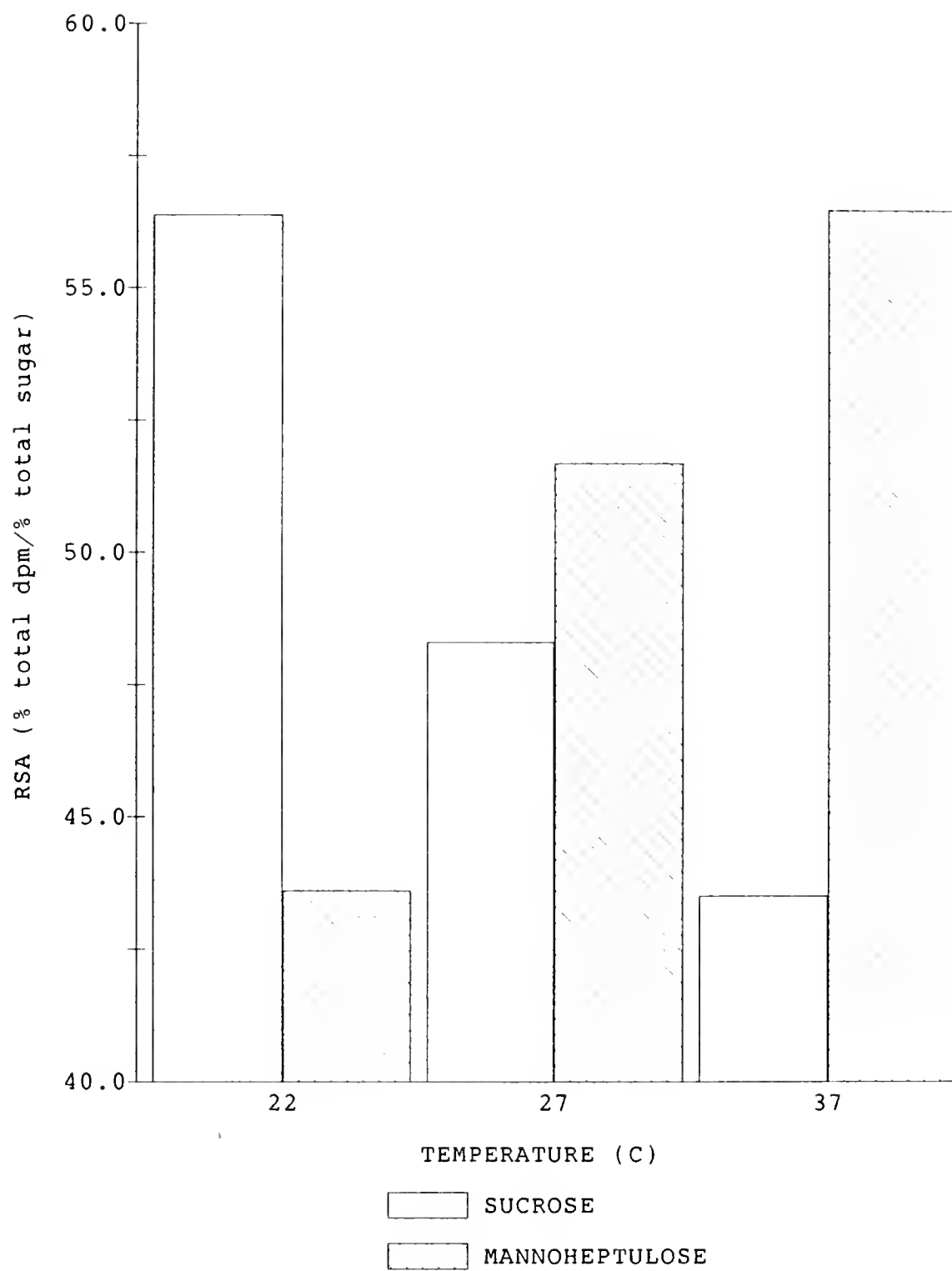
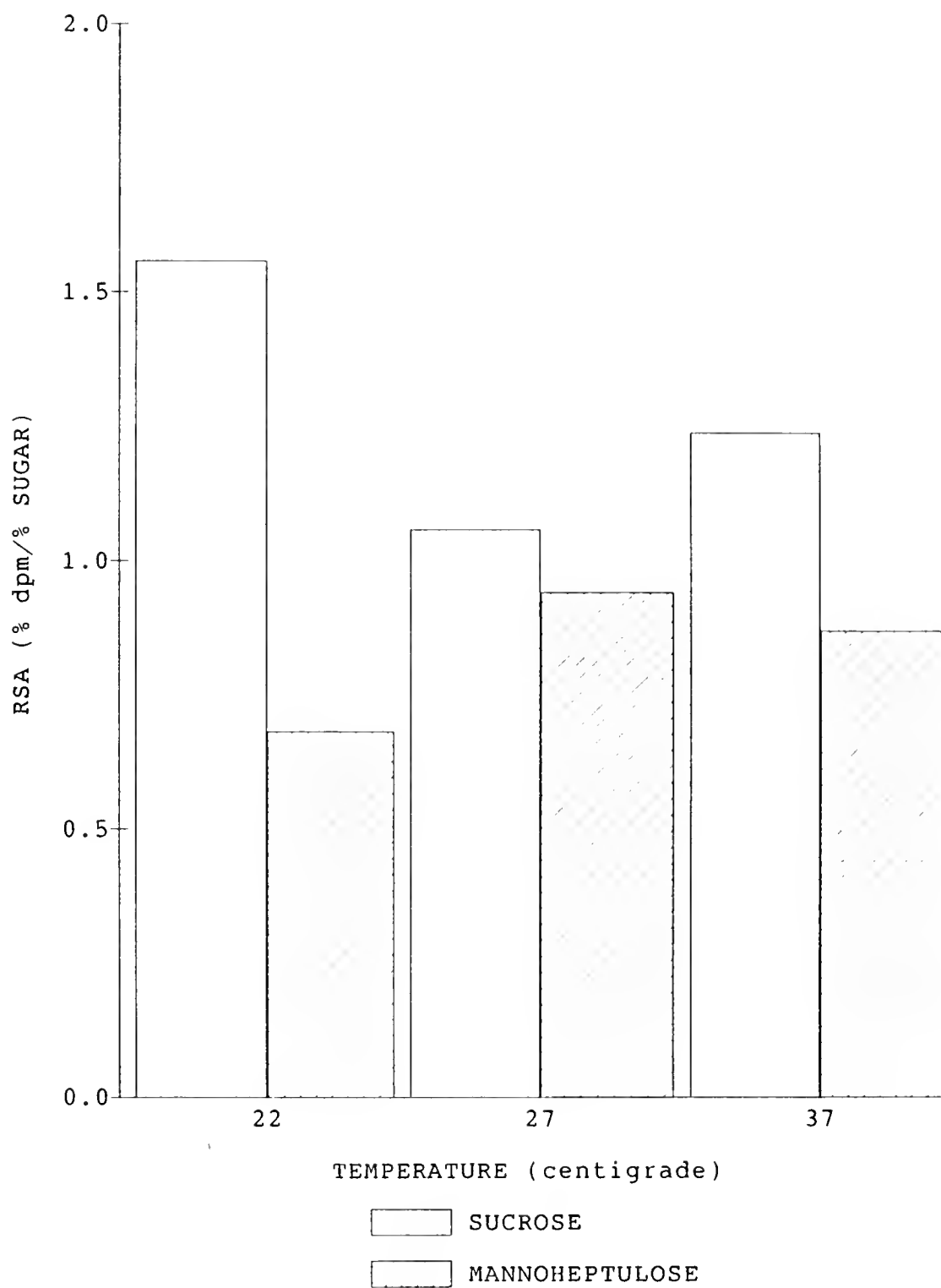


Figure 2-4. The Relative Specific Activity of Avocado Leaf Sugars at Various Temperatures. Leaves were incubated at three temperatures and fed $^{14}\text{CO}_2$. At the end of the incubation period the leaves were macerated and boiled in 85% EtOH. Leaf extracts were spotted on TLC plates which were developed in a chloroform:acetic acid:water solution. The plates were removed from the developing solution and scraped for sugar extraction and scintillation spectroscopy. The silica gel scrapings were extracted with 500 ul of water. Sugar content was measured by the phenol-sulfuric acid method. Radioactivity was measured by scintillation spectroscopy. The relative specific activity (% total dpm/% total sugar) describes the increase in sugar radioactivity in relation to the amount of sugar present.



CHAPTER 3 GROWTH OF ABSCISING AND PERSISTING AVOCADO FRUIT

Introduction

Although the growth of avocado fruit has been well characterized (18,80,133,167,169,194,207), little work has been done on the growth kinetics of abscising fruits. Avocado varieties exhibit one of two possible fruit set patterns (44). Cultivars which exhibit the Type II fruit set habit initially set few fruit which are retained until harvest. Type I fruit set pattern involves an initial heavy fruit set followed by an extended period of abscission. Typically up to 20% of the flowers will form fruitlets but only 1 to 7% of the fruit remain at harvest (44,148). This latter pattern is typical of the 'Petersen' cultivar used in this study and of the 'Hass' cultivar. Both are utilized in commercial production.

The causes of fruit abscission are unknown. Sedgely (172) has shown that 90% of the fruitlets which abscise within one week of flowering were unfertilized. Fruitlets which abscised after this period were fertile and anatomically normal (166,187,188,194). Recent studies (48,74) have suggested that genetic selection and outcrossing may play an important role in determining which fruitlets abscise.

Abscission of avocado fruitlets is characterized by the evolution of ethylene and the deterioration and browning of the seed coat (18,47) in an otherwise anatomically normal fruitlet. Davenport and Manners (47) have shown that the burst of ethylene produced by the seedcoat

occurs with the onset of nucellar senescence approximately 30 hours after excision from the tree. Exposure of excised fruit to aminoethoxyvinyl glycine did not affect the progression of senescence, but did delay fruit separation. They suggested that ethylene generated by the seed coat is essential for the activation of the cells in the abscission zone, but does not initiate senescence. An as yet undetermined event initiates seed coat senescence which in turn stimulates ethylene evolution and fruit separation.

Abscising and retained fruit may differ significantly in their development. Zucconi et al. (212) have shown that 'Shamouti' oranges stop growing 1-2 weeks before fruit separation. Similarly, the growth rate of peaches also decreased 10-20 days before abscission. The time span between growth termination and the abscission of avocado fruitlets suggested in the literature ranges from 4 days to 3 weeks (1,47,74). Adato and Gazit (1) suggested a 3 week timespan between peak ethylene production and fruit abscission. Sedgely (172) has suggested that seed coat degeneration occurs during the 7 to 8 day period between the cessation of growth and the separation of fruit from the tree. Davenport and Manners (47) however have shown that senescence of the nucellus and integuments is first visually noted within 27 hours after excision from the tree and abscission of the fruit occurs 2 days later. The overall goal of this research was to clarify the mechanism underlying the extensive, premature fruit drop in avocado. The growth of abscising and retained fruit was examined, to determine if abscising and retained fruit have inherently different growth patterns. Secondly, fruit growth in relation to abscission was used to establish a time frame for fruit abscission.

Materials and Methods

Avocado (Persea americana Mill. cv 'Petersen') trees used in these experiments were 10-year-old grafted trees growing in Rockdale soil at the Tropical Research and Education Center, Homestead, FL. Individual fruit were tagged during February and March of 1986. Tagged fruit were less than 1 cm in diameter. Fruit diameters were measured at their widest point with a fruit caliper accurate to 0.1 mm. They were measured every other day between 0830 h and 1030 h. Fruit were gently pulled before they were measured to determine if the fruit removal force had decreased. Fruit which easily separated from the tree when pulled were considered abscised. Fruit comparable in size to the tagged fruit were harvested twice during the season to measure diameter and dry mass accumulation.

The number and arrangement of fruit on each inflorescence was noted. A total of 54 inflorescences (Table 3-1) were observed during the growing season. There were 32 singly-fruited inflorescences. There were 22 multiple-fruited inflorescences bearing a total of 74 fruit.

The growth of avocado fruits has been measured directly by displaced volume (18,133), dry mass (11) and diameter (18,91,171,207). Combinations of these parameters are commonly used since the avocado has an irregular shape which cannot be easily described by a common geometric form. Because 'Petersen' is nearly spherical in shape, volume was calculated from the measured diameter using a spherical model. The actual volumes of the fruit remaining at harvest were measured by water displacement. Actual measurements did not vary by more than 6% from those calculated using the measured diameter and a spherical model.

The following equations were used to describe growth, where V represents volume:

$$\text{Relative growth rate (RGR)} = 1/V * dV/dT = f'_v(T)$$

$$\text{Absolute growth rate (AGR)} = dV/dT = f'_v(T) * \exp[f_v(T)]$$

The RGR was determined in reference to a measurable attribute V (volume) which is a function of time, $V = f(T)$. The RGR therefor may be expressed as; $RGR = 1/V^2 * [(V_2 - V_1) / (T_2 - T_1)] = f'_v(T)$ or $d \ln/dT * f'_v(T)$. The RGR describes the rate of deposition of matter in relation to the bulk of existing tissue or the rate of growth in size divided by the size of the fruit. The absolute growth rate is also a function of a measurable parameter and time $V = f(t)$. The first derivative of this function dV/dT is the AGR which describes the absolute increase in mass per unit time. AGR is the first derivative or slope of the function describing the function of time and volume and the RGR is this quantity divided by the final V.

Results

'Petersen' exhibited a single sigmoidal growth curve (Fig. 3-1). Fruit volume increased from .015 cm³ at anthesis to greater than 400 cm³ at harvest 175 days post-anthesis. Diurnal fluctuations in fruit volume due to water stress and transpiration have been observed (171); however, these effects were minimized in the present experiments by using drip irrigation and by measuring the fruit early in the morning.

Fruit volume and the dry mass (Fig. 3-2) increased rapidly and then leveled off as the season progressed. The relationship between fruit dry mass and volume (Fig. 3-2) indicated that fruit underwent substantial relative dry mass accumulation during the initial

phase of development which lasted approximately one month at which time the fruit volume was less than 4 cm^3 (Fig. 3-1). The final phase of development, which was characterized by large increases in volume and smaller increases in dry mass, began approximately 1 month post-anthesis and lasted until harvest.

Relative growth rates were calculated using natural log transformations of the data after the manner of Hunt (106). The RGR (Fig.3-3) was highest during the early season having a maximum value of $.18921 \text{ cm}^3 \text{ cm}^{-3} \text{ d}^{-1}$ and lowest late in the season with a value of $.015034 \text{ cm}^3 \text{ cm}^{-3} \text{ d}^{-1}$. The RGR describes the accumulation of volume in relation to the bulk of the tissue. Defined in terms of RGR, the fruit grew more rapidly immediately after anthesis than later in the season; however, an opposite statement can be made in terms of AGR.

The AGR increased from 0 to approximately $.2 \text{ cm}^3 \text{ d}^{-1}$ during the first month post-anthesis. Between 30 and 110 days post-anthesis the AGR increased and reached a maximum value of $2.2897 \text{ cm}^3 \text{ d}^{-1}$. The AGR decreased between 110 days post-anthesis and harvest. The curve of the AGR suggested that fruit growth occurred in 3 phases; the initial phase (0-30 days), the second phase (30-110 days) which was characterized by a linear increase in growth rate and the final phase in which a linear decrease in the growth rate was observed. The initial phase ended when the fruit were about 2 cm in diameter. The fruit increased in diameter from 2 to 6 cm during the second phase and from 6 to 11 cm in the final phase.

The arrangement and number of fruit on a branch did not affect the growth rate or fruit abscission. Frequently, fruit of similar size were retained on the inflorescences (Fig. 3-4). The growth curves of the

five fruit (86-89) shown in Fig. 3-4 indicated that growth of individual fruits on a branch was identical even though these fruit had different phyllotaxy's and different arrangements on the inflorescences (three separate inflorescences; two inflorescences bearing two fruit and one inflorescences bearing one fruit).

The growth rates (AGR) of the five fruit mentioned above were nearly identical to the growth rates observed for other fruit present singly on different branches (Fig. 3-5) or on multiple-fruited branches (fruit 86-89, fruit 76-77). The growth of abscising fruit (Fig. 3-5) was similar to retained fruit until $4.54 \pm .35$ days before abscission. Four to 5 days before fruit separation the AGR of the fruit destined to abscise decreased (data not shown). Generally fruit continued to increase slightly in volume until separation, however occasionally fruit shriveled (fruit 17) before separation.

Nearly 80% of the tagged fruit abscised during the experimental period (Fig. 3-6). The majority of these fruit (51%) were less than 1 cm in diameter. The number of fruit retained (Fig. 3-7) showed an exponential decrease over time. A large number of fruit abscised early in the season. Progressively fewer fruit abscised with time.

Discussion

Early workers in avocado growth and development determined that avocado exhibited single sigmoidal growth kinetics. These worker also showed that somewhat uniquely avocado fruit growth resulted from continuous cell division and expansion throughout development (167,169,194). The growth of the 'Petersen' cultivar can also be

described as a single sigmoidal curve. However, the relationship between fruit dry mass and fruit volume (Fig. 3-2) suggested that avocado exhibited a normal fruit growth pattern, i.e., initial growth during the first month post-anthesis was due primarily to cell division and later growth was due to cell expansion. Similar conclusions were drawn by Kushan et al. (122) from their examinations of DNA and polyamine content during fruit development of another common Florida cultivar, 'Simmonds'. They found that the DNA and polyamine content of the avocado fruit peaked at one month from full bloom which is the stage at which we observed the change in growth phases. This pattern of DNA and polyamine accumulation is similar to that observed in ovaries exhibiting a normal growth pattern .

Fruit growth rates were dependent on the stage of development. Fruit on different branches (Fig. 3-4 and 3-5) grew at the same rate at a given developmental stage. As expected the RGR values were greatest immediately post anthesis and the AGR values were greatest at mid-season. There was no difference; however, in the AGR or RGR values for abscising and retained fruit until 4-5 days prior to abscission. At this point growth rate of abscising fruit decreased. In some cases fruit volume increased marginally until fruit separation. In others, fruit volume decreased slightly before separation.

Fruit load on a branch did not affect fruit growth rates. Fruit on multiple-fruited branches (Fig. 3-4) grew at the same rate as fruit of similar size on singly-fruited branches (Fig. 3-5). In fact, phyllotaxy which strongly governs assimilate movement in avocado (Chap 7), had no

effect on the growth of fruit. Fruits on multiple-fruited branches all grew at the same rates even with different phyllotactic relationships to the source leaves.

Competition between fruits for available assimilates appeared to have very little influence on abscission in avocado. Hypothetically, a single fruit on an inflorescence should have a greater likelihood of reaching maturity since it is the sole recipient of assimilates. Conversely, competition should exist on multiple-fruited inflorescences. However the probability (Table 3-1) of abscission for single fruit (75%) is just as great as for fruit present in pairs (83%) or for individual fruit (79.5%) on a multiple-fruited inflorescence. Greater than 50% of the abscised fruit were less than 1 cm in diameter. The abscission of many of the fruitlets less than .5 cm could possibly be attributed to infertile or abnormal ovules (172). The causes of larger fruit abscission are still unknown, although it is apparently a "whole tree" and not a "local" phenomenon.

Abscising avocado fruit were indistinguishable from retained fruit until 4 to 5 days before separation, unlike orange and peach fruits (212) where growth ceased 20-30 days before separation. Furthermore, according to the literature available on avocado fruit abscission, fruit growth ceases anywhere between 4 days to 3 weeks prior to separation. This paper supports a shorter period between growth cessation and fruit separation and agrees with the abscission kinetics observed by Davenport and Manners (47). They suggested that the cessation of growth results from the ongoing process of abscission and not vice versa. Other work from this laboratory (Chap. 4) on carbohydrate availability and restriction during abscission indicated that recent photoassimilates

partitioned normally into fruit which abscised 7 or more days after labeling. Radiolabeled assimilates were detected in fruit abscising between 4 and 7 days post-labeling although the distribution of label to the fruit tissues differed from control fruit. No label was recovered from the tissues of fruit which abscised within 4 days of labeling. This suggests that the initiation of senescence altered fruit assimilate import during a 4 to 7 day period prior to abscission; however, growth rates did not decrease until 4 to 5 days before fruit separation. The restriction of assimilate movement to fruit at 4 days before fruit separation agrees with our fruit growth data and supports the contention of Davenport and Manners that the cessation of fruit growth is due to the initiation of senescence and its accompanying phenomena.

This study was part of a larger effort to understand carbohydrate availability and abscission in avocado. Abscising fruit were indistinguishable from retained fruit until 4 to 5 days before fruit separation. We suggest that fruit abscission resulted from an initial senescence event perceived by the fruit 5-7 days before actual separation. This event initiates nucellar senescence and ethylene evolution. Ethylene induced cell division in the abscission zone restricted the movement of assimilates and water into the fruit causing a decrease in the rate of growth. Cessation of fruit growth therefore was the result and not the cause of abscission. Local source limitation also did not appear to be a likely factor in fruit abscission since the probability of abscission was the same for all fruit whether they were present on a branch singly, in pairs, or other multiples. Finally, although cv. 'Petersen' exhibited a single sigmoidal growth curve

typical of avocado, the mass and volume data suggested it follows a normal pattern of growth and did not exhibit cell division and expansion throughout development.

Table 3-1. Abscised Fruitlets on Singly-fruited and Multiple-fruited Inflorescences.

Inflorescences bearing a total of 106 fruit were monitored between February and August of 1986. There were 22 multiple-fruited inflorescences bearing a total of 74 fruit. Of these 22 inflorescences, 15 bore at least two-fruit each on a single peduncle. In the table below, "pairs" refers to two fruit borne on the same peduncle while "individual" refers to two or more fruit on the same inflorescence but borne on different peduncles. Pairs and individual fruit did exist on the same inflorescence. A total of 32 inflorescences bearing only one fruit were observed. These fruit are referred to as single fruit in the table below. The heading "% of abscised fruit" refers to the abscised fruit within each of the following categories; pairs, individual or single classifications of fruit. A total of 84 fruit abscised during the period of observation.

Inflorescence Type	Total # of Fruit Examined	% of Abscised	% of Total Abscised	% of Total Fruit
<hr/>				
<u>Multiple-fruited</u>				
Pairs	30	83*	29.8	23.1
Individual	44	79.5*	41.6	32.4
<u>Single-fruited</u>				
Single	32	75*	28.6	22.2

* At a 95% confidence level these % are not significantly different.

Figure 3-1. Growth Curve for Persea americana Mill. cv. 'Petersen'. This curve is a composite of sequential non-destructive data from at least 10 non-abscising fruit. Volume was calculated from the measured diameter using a spherical model. Volumes were transformed to their natural log values. The slope of this curve $d \ln V/dT$ is the RGR.

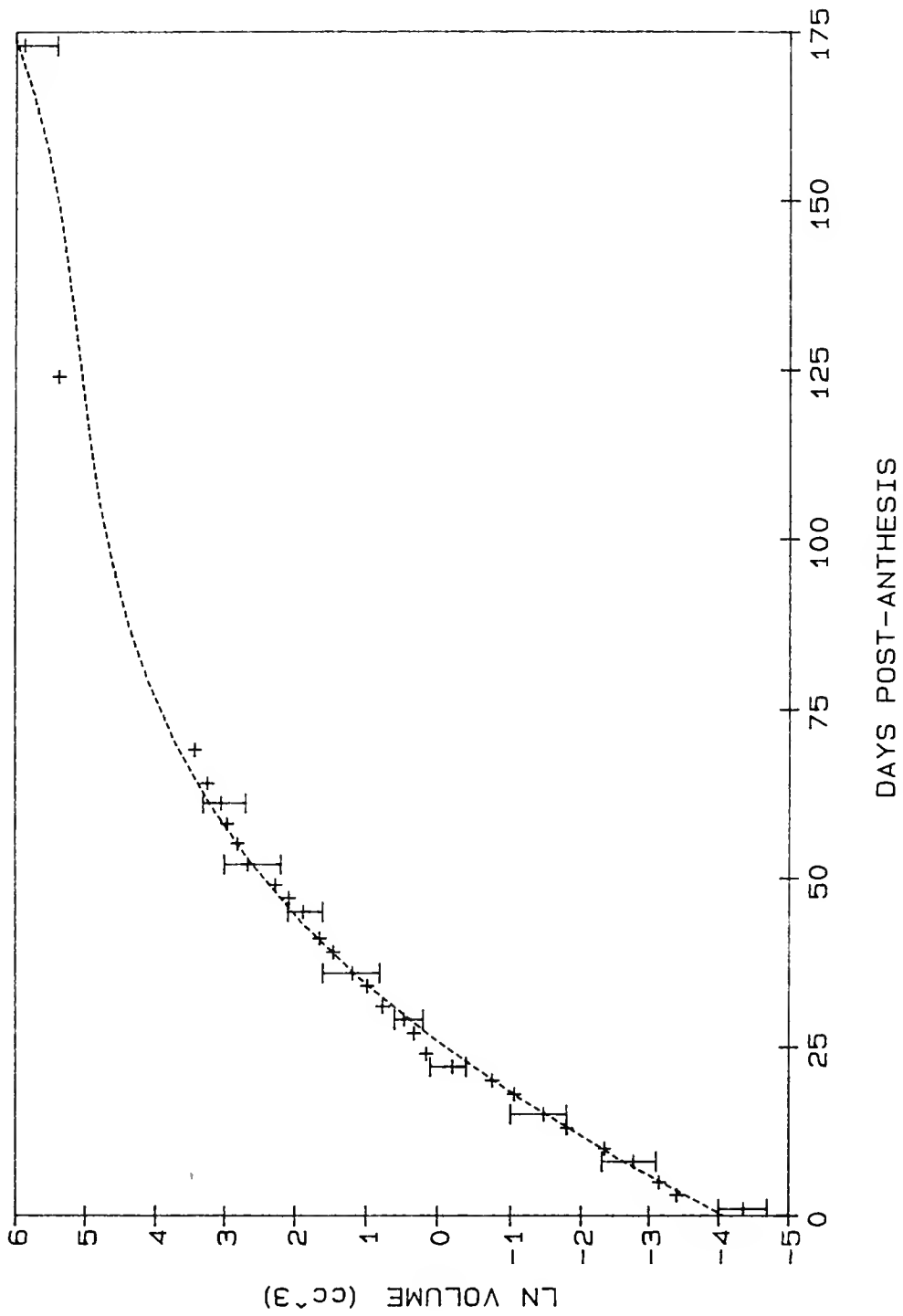


Figure 3-2. Volume vs Dry Mass of Avocado Fruit. Avocado fruits were harvested periodically during the season for dry mass measurements. The increase in mass with diameter paralleled the increase in volume predicted by a spherical fruit model. Each point represents a single measurement.

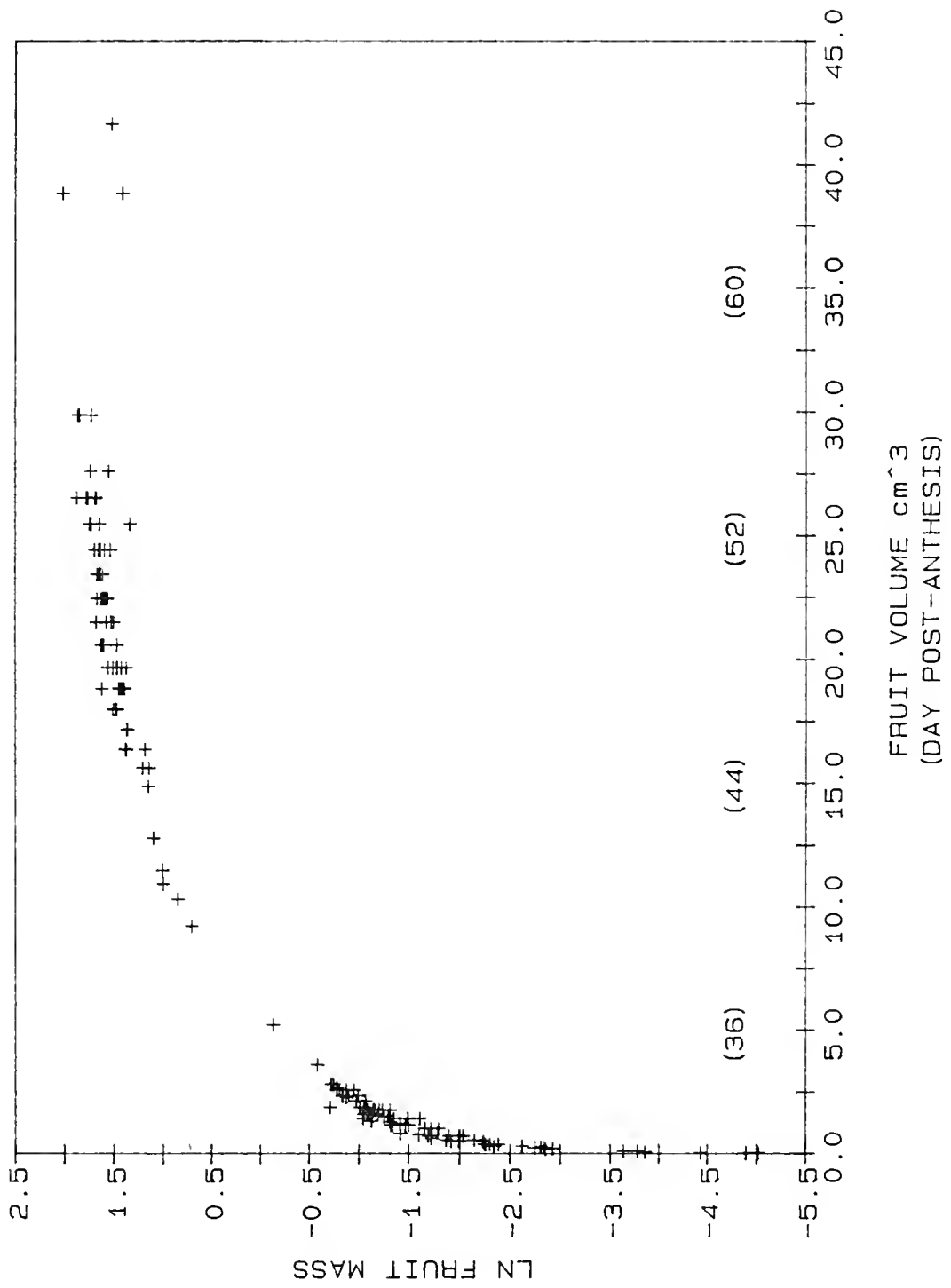


Figure 3-3. Absolute and Relative Growth Rates of Avocado. The AGR and RGR were calculated after Hunt (106). The maximum AGR was $2.2897 \text{ cm}^3 \text{ d}^{-1}$ at 110 days post anthesis. The RGR was greatest ($.18921 \text{ cm}^3 \text{ cm}^{-3} \text{ d}^{-1}$) early in the season.

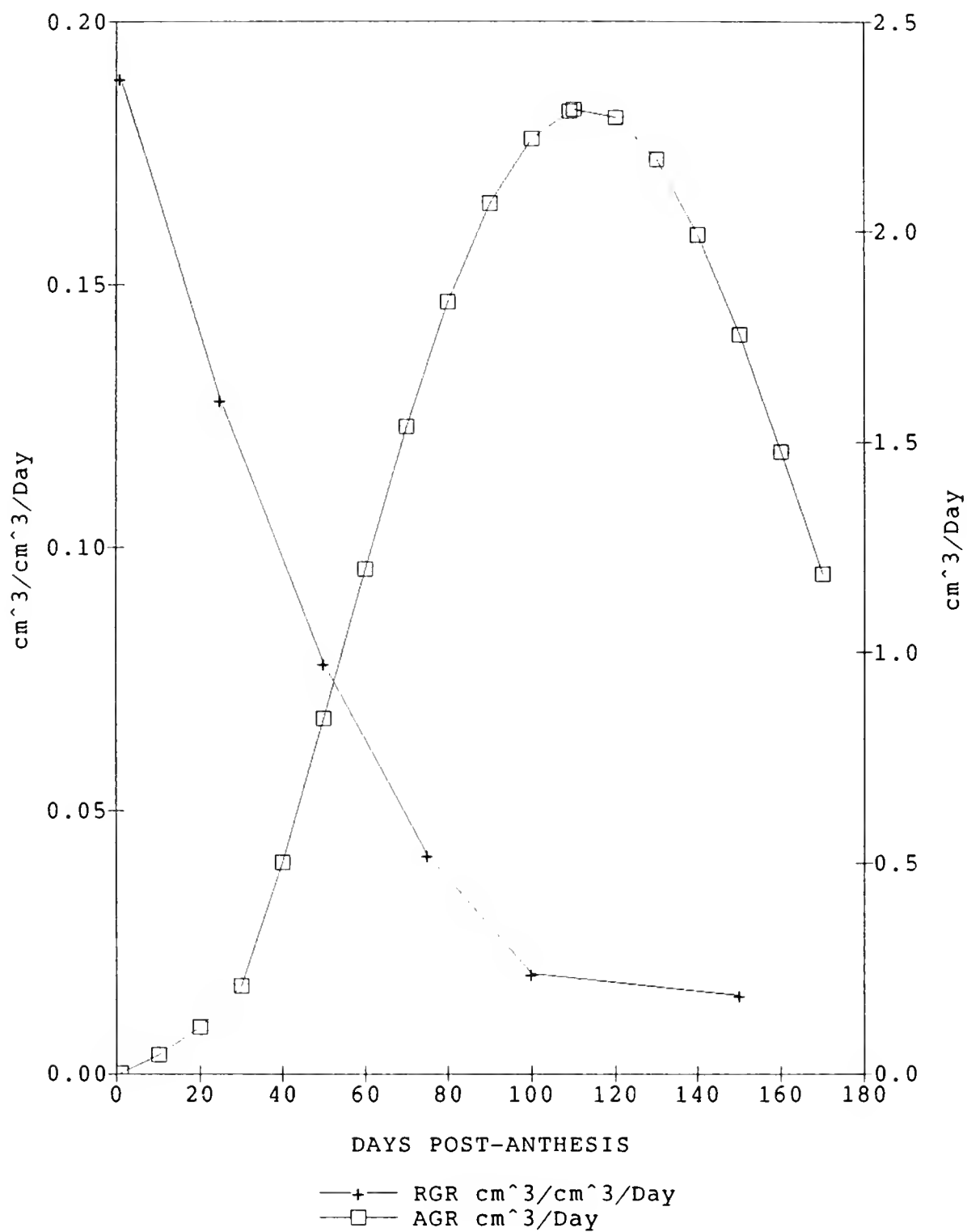
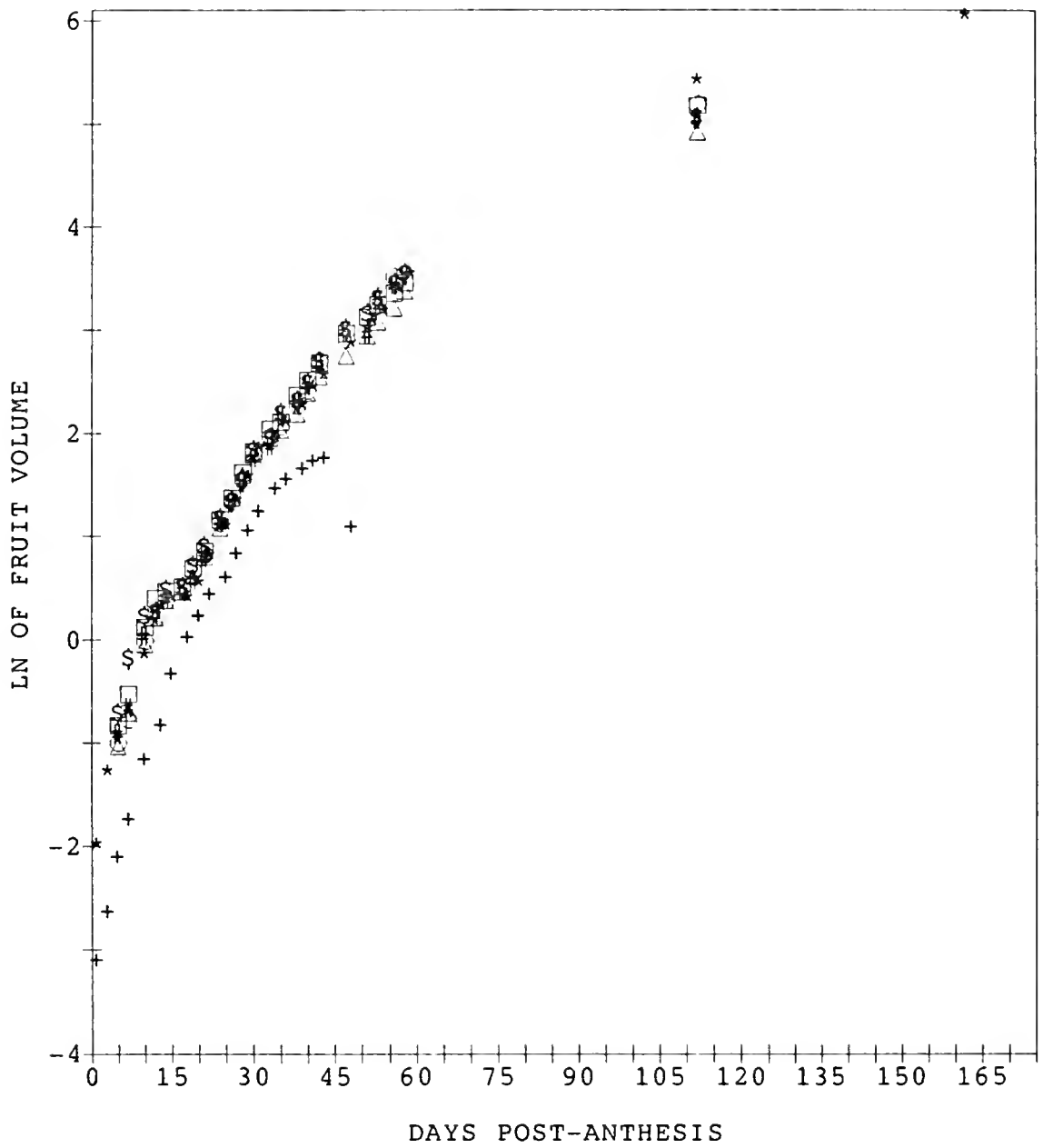
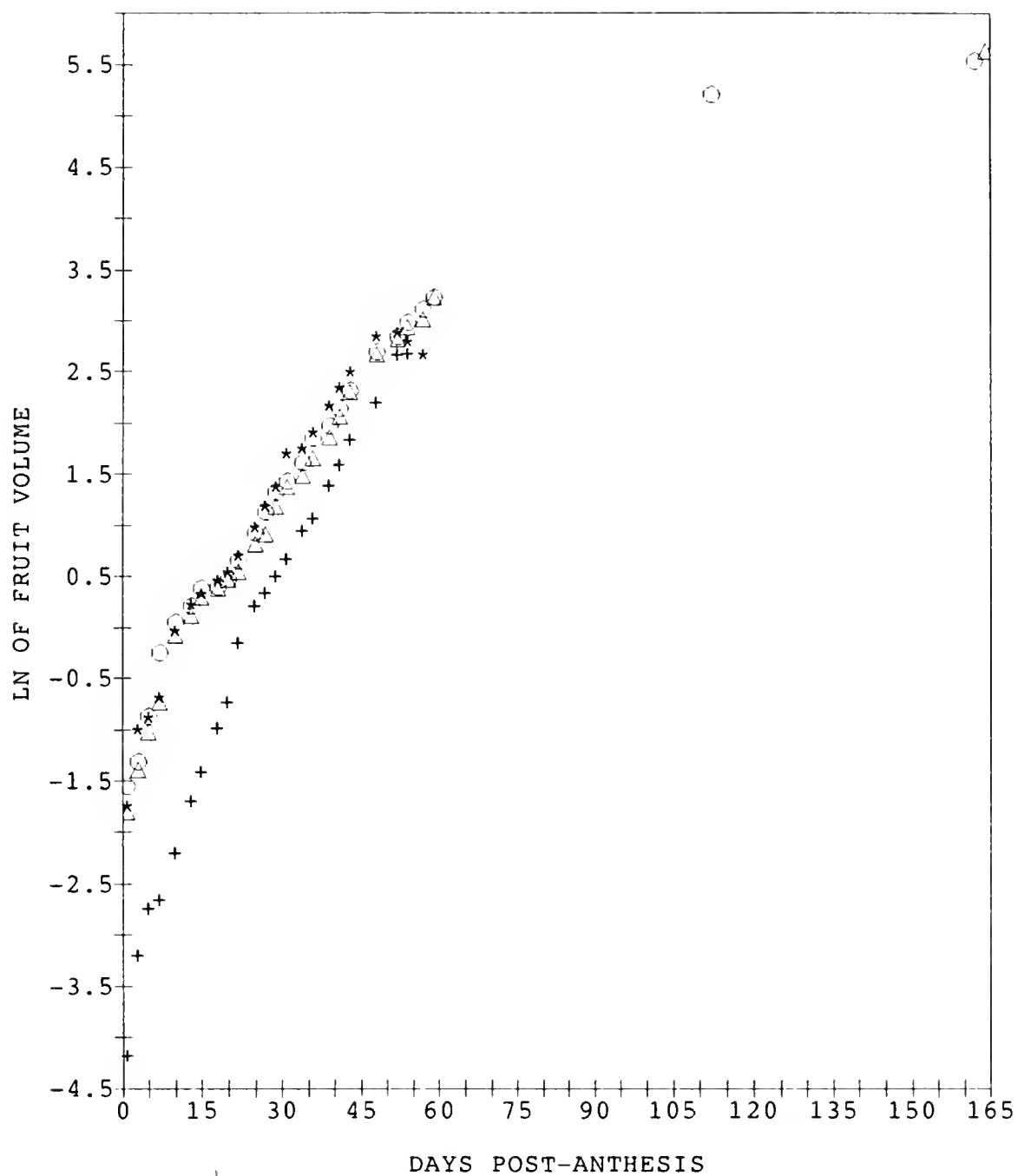


Figure 3-4. Growth of Fruit on Multiple-fruited Inflorescence. Fruit 86-89 were present on the same inflorescence and none of these fruit abscised during the course of the experiment. Fruit 76 and 77 were also present on the same inflorscence, however fruit 76 abscised after 50 days of study.



+	FRUIT # 76
*	FRUIT # 77
○	FRUIT # 86
△	FRUIT # 87
□	FRUIT # 88
#	FRUIT # 89
\$	FRUIT # 90

Figure 3-5. Growth of Individual Fruit on a Single-fruited Inflorescence. Fruit 16 and 17 abscised approximately 55 days after tagging, while fruits 21 and 27 were retained until harvest.



+ FRUIT # 16
* FRUIT # 17
○ FRUIT # 21
△ FRUIT # 27

Figure 3-6. Distribution of Abscised Fruitlets by Size. The majority of fruit which abscised had a diameter less than 1 cm. A total of 84 of the originally tagged 108 fruit abscised.

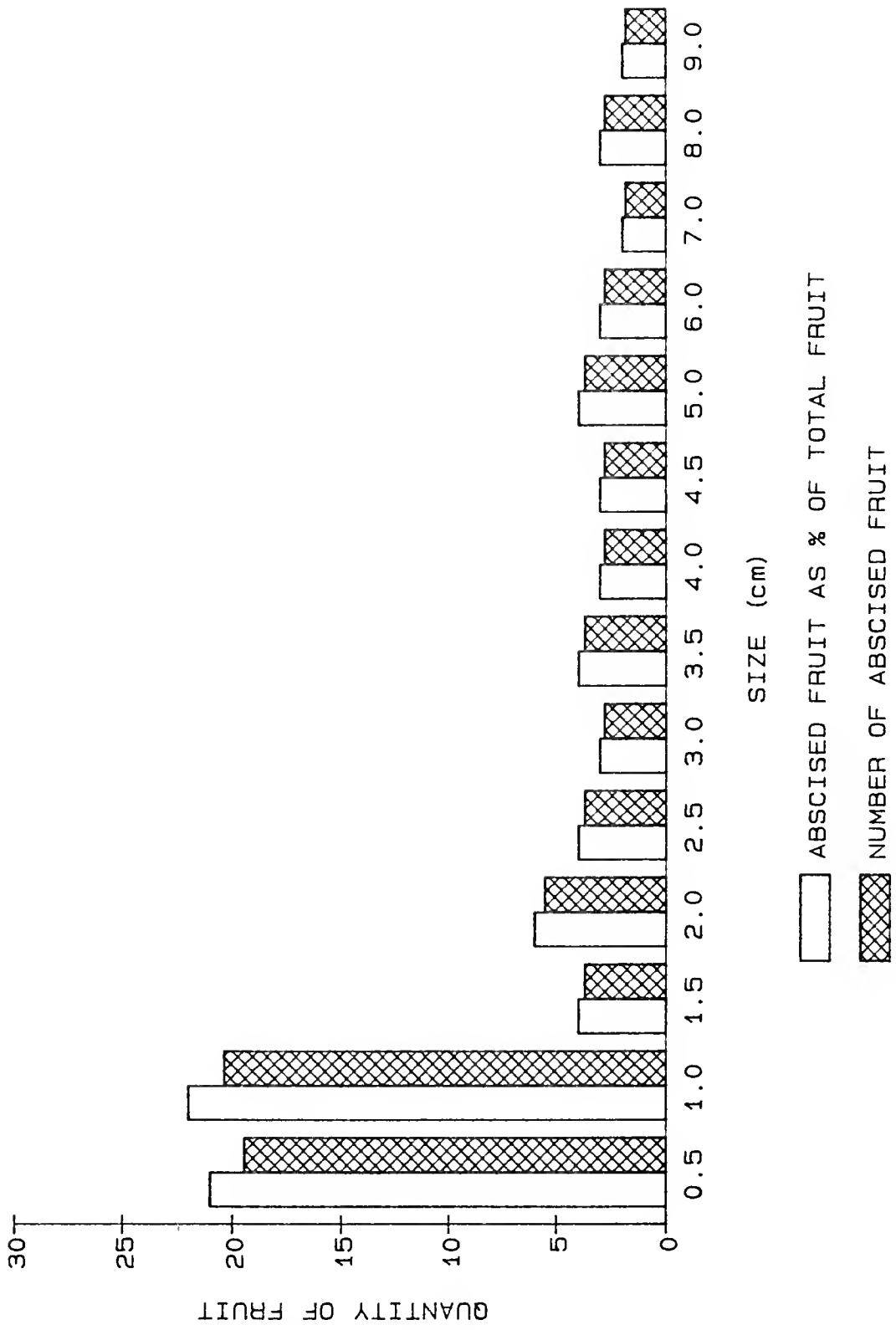
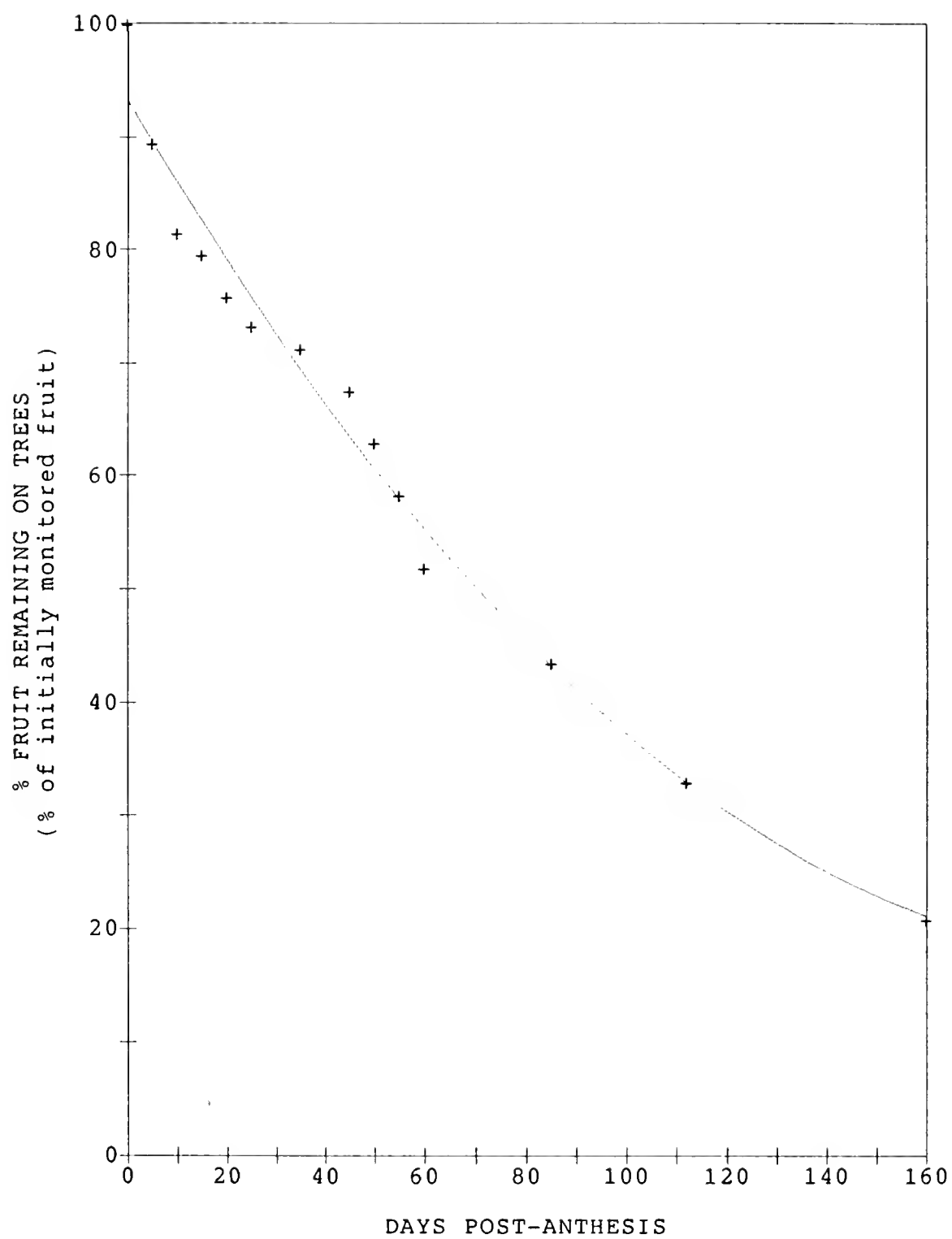


Figure 3-7. Abscission of Fruit over Time. The number of fruit remaining on the trees decreased exponentially with days post-anthesis.



CHAPTER 4
ASSIMILATE PARTITIONING BETWEEN ABSCISING AND PERSISTING AVOCADO FRUITS

Introduction

The causes of avocado fruit abscission remain unclear even though fruit loss is extensive and economically damaging. Avocado exhibits two fruit set patterns. In the Type II pattern very few fruit are initially set; however, these fruit are retained until harvest. In the Type I fruit set pattern a heavy fruit set is followed by a prolonged period of extensive fruit abscission. This pattern is typical of many commercial varieties and the 'Petersen' cultivar used in this study. Sedgely (172) has shown that 90% of the fruitlets which abscise within one week of flowering were unfertilized. Fruitlets which abscised after this period were fertile and anatomically normal (172,187,188). Recent studies (48,74) have shown that genetic selection and outcrossing may play an important role in determining which fruitlets abscise. Abscission of fruitlets can also be induced by injuring or puncturing the seed coat (19).

The suggested time frame in the literature for the senescence and abscission of fruitlets ranges from 4 days to 3 weeks. Abscission of avocado fruitlets is characterized by deterioration and browning of the seed coat (18) in an otherwise anatomically normal fruitlet. Adato and Gazit (1) suggested that the seed coat degeneration was related to increased ethylene production. This in turn correlated with fruit abscission. They suggested a 3 week timespan between peak ethylene

production and abscission. Sedgely (172) suggested that seed degeneration occurs during the 7 to 8 day period between the cessation of growth and the separation of fruit from the tree. Davenport and Manners (47) showed that senescence of the nucellus and the seed coat are relatively rapid events. Senescence of the nucellus and seed coat was first visually noted within 33 hours after excision of the fruit-bearing branches from trees, and abscission of the fruit occurred 2 days after the onset of senescence. Accordingly, they concluded that seed coat senescence occurs early in the scheme of events and plays an integral role in abscission. Ethylene production which is a product of the senescence process is responsible for the induction of flower and fruitlet abscission.

Work in this laboratory indicated that fruit which abscised within 4 days of source leaf labeling ($^{14}\text{CO}_2$) contained no radiolabeled assimilates. Fruits which abscised between 4 and 8 days after source leaf labeling contained radiolabel; however, a greater percentage of the total label was recovered in the seedcoats of abscised versus non-abscised fruit. The distribution of label in fruits which abscised after 8 days was identical to the non-abscising controls. In this study the abscised fruit exhibited seed coat discoloration but no other damage or gross anatomic abnormalities were evident.

This research was part of a larger effort to delineate the mechanisms of the extensive and premature abscission of avocado fruit. The specific objectives of this research were two-fold. First, the accumulation of labeled recent photoassimilates by the tissues of control and puncture-induced-abscising fruit will be compared to

determine if recent photoassimilates distribute differently within the fruits during abscission. Second, individual fruit will be punctured at time intervals before and after source leaf labeling in order to establish a time frame for the restriction of assimilate movement, senescence and abscission.

Materials and Methods

Fruit Labeling

The trees in this study were ten-year-old 'Peterson' orchard trees growing on Walden rootstock at the Tropical Research and Education Center in Homestead, FL.

Branches (approximately 1 cm in diameter) were girdled 24 hours before labeling. Individual leaves were enclosed in Plexiglas containers and fed with 20 uCi of $^{14}\text{CO}_2$ generated from $\text{NaH}^{14}\text{CO}_3$ (Amersham with a specific activity of 54 m Ci/mmol) using an equimolar amount of 5% lactic acid. After 1 hour the containers were removed and two disks ($.309 \text{ cm}^2$) were taken from the source leaves using a hole punch. Source leaves were subsequently sampled twice daily for the remainder of the experiment. Fruit diameters were measured twice daily as described previously (Chap. 3). Growth rates were calculated after the method of Hunt (106) and as described in Chap. 3.

Partitioning Studies

Source leaves were labeled as described above. After 48 hours the branch (1-2 cm in diameter, 30-45 cm in length) bearing the fruit was removed. The fruit were dissected with a sharp knife and forceps and individual tissues dried at 56°C . The dried tissue was weighed, digested, and decolorized by the method of Burrell and Brunt (25) in preparation for scintillation counting.

Puncture Studies

The term, 'single fruit' refers to an inflorescence bearing only one fruit. Similarly, doublet fruit refers to 2 fruit sharing the same inflorescence. Six single fruit and 6 doublet fruit were used in each experiment, which was repeated 4 times.

Fruit were pierced deeply enough to injure the seed coat using a stainless steel wire (gauge #20) either before (24,48,72 and 96 hours), after (24,48,72 and 96 hours) or at the time of source leaf labeling (0 hour). This technique allowed us to arbitrarily induce abscission of individual fruitlets. Both the fruit and the wire were surface sterilized with dilute chlorox solution before and after puncturing the seed coat. Only one fruit on the inflorescence was punctured when two were present.

Fruit Growth

Fruit were measured twice daily at their widest point with a fruit caliper.

Results

The vascular system of avocado is asymmetric (19,41) and gradients of dry mass and oil content in the fruit have been noted (170). These gradients are oriented radially around the seed and longitudinally from the stem to blossom end. Recent photoassimilates were distributed symmetrically throughout the radial and longitudinal quadrants of the fruit (Table 4-1). The data were obtained from fruits with different amounts of dry mass and different source leaf-to-fruit positions. The

variations observed in the specific activities of tissues between and within fruits corresponded to developmental and phyllotactic differences.

Piercing the fruit did not affect the translocation rates of assimilates from source leaves during the course of the experiments. An average of 33.3 +/- 18% of the initially fixed dpm were recovered in the fruit 96 hours after labeling. This equates to 48.7 +/- 24.3% of the total translocated dpm. Individual fruit averaged 1.9×10^6 +/- 1.6×10^6 dpm. The large standard deviation reflects the strong effect of phyllotaxy on the partitioning of recent photoassimilates (Chap. 7). For example if the source leaf and fruit had similar phyllotaxy then nearly 95% of the translocated label was recovered in that fruit. However, if the fruit and source leaf were not in direct alignment only a small percentage of label would move into the fruit (Chap. 7). Since source leaves were labeled without regard to phyllotaxy, a large discrepancy in the absolute imported dpm's was observed.

Relative assimilate import by the tissues of different sized fruits is shown in Table 4-2. The numbers given under each tissue type represented the percentage of the total fruit in which that tissue had the greatest specific activity. For example, the endosperm had the greatest tissue specific activity in 51.3% of the fruit in the 0-0.5 gms dry mass range. In approximately 5% of the fruits with dry masses less than 1 gram, the seed coat was the most actively importing tissue. Fruitlets in this size range also composed the greatest percentage of abscised fruit (Chap. 3). Fewer fruit in the 1 to 3 gram range abscised, and interestingly the seed coats of fruit in this sample were

not the most actively importing tissue. Generally, the endosperm was the most active importer of assimilates, but since it represented such a small percentage of the dry mass its sink activity was overshadowed by the more massive tissues.

The total amount of radiolabel in a tissue appeared to be a function of mass (Table 4-3). For example, the mesocarp comprised between 87 and 89% of the fruit dry mass for fruit with a total dry mass less than 1.5 grams. The mesocarp also imported 84 to 88% of the total radiolabel recovered from the fruit. Similarly, the seed coat comprised 7.4 to 9% of the fruit dry mass and imported 8.6 to 9.7% of the total radiolabel recovered from the fruit. Although all of the fruit tissues are importing radiolabel, the endosperm and cotyledons have a high ^{14}C /dry mass ratio indicating these tissues are growing more rapidly than the mesocarp and seedcoat.

Fruit were pierced to initiate seed coat senescence and abscission (19). The distribution of radiolabel in the tissues of pierced, single fruits is shown in Fig. 4-1. In the non-pierced control the label distribution was as described in Table 4-3. The mesocarp contained approximately 88% of the total label recovered from the fruit. In fruit pierced 48 h before source leaf labeling only about 52% of the total label recovered from the fruit was found in the mesocarp. The percentage of label found in the seed coat increased and was greater than 5-times the percentage found in control fruit. In fruit punctured 24 hours before source leaf labeling, the percentages of label recovered in the endosperm, cotyledons and seedcoat were significantly greater and the percentage of label recovered in the mesocarp was significantly less

than in the control fruit. Fruit pierced at the same time or 24 to 48 h after labeling had a greater percentage of label in the mesocarp than did control fruit. Data for fruit punctured 72 and 96 hours before labeling are not shown. These fruit abscised soon after labeling and contained no radiolabel. Data for fruit punctured 72 and 96 hours after labeling are also not shown. The relative percentage radioactivity in these fruit did not vary from control values.

The distribution of radiolabel in the pierced fruitlet on the double-fruited inflorescence is shown in Fig. 4-2. Data for the unpierced fruitlet were identical to the control.

In single fruit the percentage of radiolabel was significantly lower in the mesocarp and higher in the seed coat than in the control when the fruit was punctured 48 or 24 hours before labeling. The percentage of radiolabel was higher in the cotyledons and endosperm than in the control tissues if punctured 48 and 24 hours before labeling and lower than the control when the fruit was pierced at 0, 24 or 48 hours post-labeling. Puncturing the seed coat of a single fruit on an inflorescence decreased its growth rate by 92%. The growth rates of both fruit on the doubly-fruited inflorescence were affected when only one was punctured. The growth rate of the punctured fruitlet decreased 50%, while the growth rate of the non-punctured fruitlet decreased 36%.

Discussion

The anatomy of the developing avocado fruit has been described by Cummings and Schroeder (41). The avocado seed develops off center producing a thin and a thick side to the fruit. The six major strands of vascular tissue are asymmetrically distributed (19,41). The vascular

strands on the thicker side enter and anastomose through the entire seed coat. The vascular strands on the thinner side anastomose through the pericarp. The asymmetry in the vascular system may explain the gradients in oil content (170) and dry weight (170) observed in the fruit; however, we found the specific activity (Table 4-1) of radiolabeled assimilates in each tissue was consistently similar throughout the tissue. This discrepancy between oil and dry mass content and radiolabel distribution results from the dependency of specific activity measurements on dry mass. As can be seen in Table 4-3 the distribution of ^{14}C assimilates paralleled dry mass content.

The import of assimilates as measured by the relative specific activity (Table 4-2) suggested that assimilation of recent photoassimilates by specific tissues depended upon fruit size. Generally, the endosperm and the mesocarp exhibited the greatest relative specific activity. The greater activity in the endosperm may correlate to this tissue's nutritive role during embryo development. The relative specific activity of the seed coat was appreciably greater than other fruit tissues only in fruits with dry masses' less than 1 gram. A large proportion of fruitlets in this size range were shown to abscise in a previous study (Chap. 3). The possible significance of this increased sink strength will be discussed later.

Inducing synchronous abscission of avocado fruitlets has been accomplished using excised fruit-bearing branches in the laboratory (47). However,¹ in order to preserve the integrity of the source/sink pathway, an alternate method was employed in the field. Puncturing the seed coat of the fruitlet as described by Blumenfeld and Gazit (18) was effective in inducing abscission. The import of radiolabeled

assimilates by fruit treated in this way mimiced the ratios found in non-punctured abscised fruitlets observed in preliminary studies (data not shown) suggesting the puncture treatment was a reasonable means of testing "natural" abscission events.

Piercing the seed coat drastically decreased the growth rate of all of the fruit on the inflorescence (Table 4-4). Cessation of growth by retained orange fruits has also been reported by Zucconi et al. (212). They found that persisting orange fruit exhibited stunted growth shortly before the peak drop of abscising fruit.

Fruitlets abscised 4 to 5 days after puncturing. The observation of diminished fruit growth by persisting fruit on inflorescences where abscission is occurring was noted in this study but not in the earlier fruit growth study from this laboratory (Chap. 3). This discrepancy is probably due to the frequency of fruit measurements. The twice daily measurements clearly showed the transient decreases in growth of persisting fruits which were not evident in the earlier study when fruit were measured every 2 days.

Although the growth rate of a non-punctured fruit on a multiple-fruited inflorescence decreased when another fruit was punctured, the presence of the non-punctured fruitlet appeared to increase the longevity of the pierced fruit. This was supported by the observation that pierced single fruitlets abscised sooner than their doublet counterparts (data not shown). Single fruitlets tended to abscise 4 to 5 days after puncturing, while their double-fruited counterparts required 5 to 6 days for separation.

The distribution of radiolabeled assimilates to the various tissues of punctured single (Fig. 4-6) and double fruits (Fig. 4-7) also differed. The anatomy of the vascular system may explain these departures from control values and provide a time frame for abscission. The mesocarp (pericarp) and the seed coat are directly supplied by vascular strands. The endosperm and the cotyledons have no direct connection to the vascular system. The relative percentage radioactivity in the mesocarp of single pierced fruitlets was significantly lower if pierced 24 or 48 hours before labeling. The process of phloem restriction therefore began within 24 hours. The higher proportion of radioactivity in the seed coat suggests that the vascular strands supplying the seed coat were not affected or not restricted to the same degree as the vascular strands supplying the mesocarp. This may result from the asymmetric distribution of the vascular strands (41) and the mass of the tissues. The vascular strands supplying the seed coat pass through the pericarp in the thicker side of the fruit. If the abscission "signal" traveled cell to cell the mass of the pericarp would hinder the movement of the abscission signal to the phloem. The vascular strands supplying the mesocarp originate from the thinner side of the fruit. These strands may perceive an abscission signal sooner than the vascular strands supplying the seed coat because fewer cells lie between the vascular strands and the putative origin of the abscission signal. The comparative mass of the tissues may also contribute to the delay observed in the restriction of the vascular strands. The less massive seed coat imports less assimilate than the more massive mesocarp. mass flow of assimilate may be easier to curtail to the seed coat than to the mesocarp.

Like the mesocarp, however, the percentage of radiolabel in the seed coat does not vary from control values if the seed coat is punctured at 0 hour or after labeling. The lower values found in the cotyledons and endosperm after labeling could be an injury response such as an increase in respiration or redistribution of the assimilates. These lower values could also be caused by restricted release of assimilates from the seed coat. Since no direct vascular connection exists to these tissues they are dependent on the release of assimilates by the seed coat into the apoplast. In any case, it was evident that puncturing the seed coat immediately set in motion processes that altered assimilate distribution to the endosperm and cotyledons independent of vascular restriction.

Similar trends were seen in the distribution of assimilates to pierced fruit on doublet inflorescences. The effects of vascular restriction seemed to occur more quickly since the puncture effects were noted even in fruit pierced 24-48 hours after labeling. Although initiation of abscission occurred quickly the completion of the process appeared delayed in multiple-fruited inflorescences.

Single fruit pierced 72 hours before labeling abscised 24 hours after labeling and contained no radiolabel. Phloem restriction was therefore complete within 72 hours; however, separation of the fruit required 24 more hours. In fact, the receptacles of fruit pierced 24 and 48 hours before labeling contained 50% as much radiolabel as their subtending fruit indicating either vascular restriction greatly increased metabolic activity in the abscission zone. Fruit pierced at 0 or 24 hours post labeling exhibited slightly

enhanced values of radiolabel in the receptacles. Fruit pierced at 48 hours post labeling showed no increase in radiolabel in their receptacles over control values.

Further insight into the physiological activity of the tissues may be gained by examining the tissue specific activity in abscised fruits (data not shown). The relative sink strength of tissues in pierced single fruitlets was similar to non-pierced controls. Similarly, doublet fruit pierced before labeling and at 0 hour had similar relative specific activities. However, in doublet fruit pierced 24 or 48 hours after labeling, the seed coat and not the endosperm/mesocarp had the greatest sink strength. The increased sink strength of the seed coat may reflect the increased activity in the seed coat which preceded abscission. This increased sink strength decreased with time after puncturing.

In conclusion, the partitioning of assimilates and the growth of abscising fruit differed from, but was influenced by persisting fruit. Punctured fruit on multiple-fruited inflorescences were retained longer than their single-fruited counterparts. Although similar trends were observed in partitioning patterns in both single- and multiple-fruited inflorescences, the differences in the latter occurred over an extended period of time. It is unknown whether or not these patterns result strictly from vascular restrictions or from other inherent metabolic processes associated with abscission. It is clear that persisting fruit do in some way influence the abscission behavior of the abscising fruit.

Puncturing the seed coat caused an immediate increase in its sink strength. This increase may reflect metabolic activity associated with the initiation of the abscission processes or an injury response.

Vascular restriction, as indicated by label accumulation in the abscission zone, began within 24 hours of seed coat puncturing. At 48 hours vascular restriction continued and seed coat metabolic activity, as measured by the specific activity began to decline. At 72 hours vascular restriction was complete. Fruit separation, however, required another 24 to 48 hours to complete.

Table 4-1. Symmetrical Partitioning of Assimilates within Tissues of Avocado Fruit.

Source leaves on branches were fed $^{14}\text{CO}_2$ for a one hour period. The fruit were harvested either 24 or 48 hours later and dissected, dried and processed for scintillation counting. Fruit represent a variety of fruit sizes (.2 - 1.2 gms.).

Fruit #	1	2	3	4	5	6
			(dpm mg^{-1} dry weight)			
Dry Mass(gm)	0.21	0.36	0.67	0.93	0.72	1.20
TISSUE						
Endosperm	2889	2889	8415	302	104	987
Endosperm	2996	2978	8422	278	100	914
Cotyledon	2300	3200	3776	894	42	916
Cotyledon	2100	3263	3985	919	44	902
Seed coat	1875	2313	2914	625	57	1631
Seed coat	1914	2266	2850	650	55	1560
Mesocarp	3818	1954	4923	1522	98	1909
Mesocarp	3209	2048	5550	1491	100	1694
Mesocarp				1464		1739
Mesocarp						1849

The data from six different fruit are presented. Replicate samples from longitudinal, radial or cross-sectional sections were tested from each tissue.

Table 4-2. Relative Specific Activities of Individual Fruit Tissues during Avocado Fruit Development.

Source leaves on branches were fed $^{14}\text{CO}_2$ for a one hour period. The fruit were harvested 24 or 48 hours later² and dissected, dried and processed for scintillation counting. Tissue specific activity is defined as dpm mg^{-1} dry mass. The data represent the percentage of the sample in which that tissue had the greatest specific activity; e.g. in fruit of the 0 to 0.5 gm range, the seed coat had the greatest specific activity in 5.3% of the fruit.

Fruit Dry Mass (gm)	# Fruit Sampled	Mesocarp	Seed Coat	Endosperm	Cotyledon
(percentage of samples in which specific activity was greastest)					
0 - 0.5	76	23.7	5.3	51.3	19.7
.51 - 1.0	49	10.2	4.1	61.2	24.5
1.01 - 1.5	15	20.0	0.0	66.6	13.3
1.51 - 3.0	8	37.5	0.0	50.0	12.5

Table 4-3. Distribution of Dry Mass and Translocated ^{14}C -Assimilates in Fruit Tissues.

Source leaves on branches were fed $^{14}\text{CO}_2$ for a one hour period. The fruit were harvested 48 hours later, dissected, dried and processed for scintillation counting. The relative specific activity (RSA) is defined as % ^{14}C / % dry mass.

Dry mass (gm)	#Fruit Sampled	Tissue	% of Total Fruit ^{14}C Recovered	% of Total Fruit Dry Mass	RSA
0 - 0.5	28	Mesocarp	87.9 \pm 5.6	87.9 \pm 2.8	1.00
		Seed coat	8.7 \pm 3.9	8.8 \pm 1.0	0.99
		Endosperm	1.9 \pm 1.0	1.6 \pm 0.7	1.18
		Cotyledon	1.9 \pm 1.6	1.4 \pm 1.0	1.35
.501 - 1.0	20	Mesocarp	87.4 \pm 5.2	89.4 \pm 0.9	0.98
		Seed coat	8.6 \pm 3.5	7.4 \pm 0.7	1.16
		Endosperm	1.3 \pm 0.7	0.9 \pm 0.3	1.44
		Cotyledon	2.7 \pm 1.4	2.3 \pm 0.7	1.17
1.01 - 1.5	4	Mesocarp	83.5 \pm 4.2	88.3 \pm 1.6	0.95
		Seed coat	9.7 \pm 3.6	7.9 \pm 0.8	1.23
		Endosperm	1.9 \pm 0.4	1.1 \pm 0.3	1.72
		Cotyledon	4.6 \pm 1.3	2.9 \pm 0.4	1.58

Table 4-4. Fruit Growth

Fruit were measured twice daily at 0800 and 1600h with a fruit caliper. Fruit volume (cm^3) was calculated using a sperical model as described in Chap. 3.

Experiment 1: Average increase in volume before piercing.

Experiment 2: Average increase in volume after piercing.

	SINGLE	DOUBLE (PIERCED)	DOUBLE (NOT PIERCED)
Experiment			
1	13.50 \pm 6.79	5.69 \pm 4.84	9.11 \pm 8.40
2	1.11 \pm 5.26	2.85 \pm 4.13	3.32 \pm 4.67

Figure 4-1. Distribution of ^{14}C in Pierced Single Fruits. Source leaves on branches bearing one fruit were fed $^{14}\text{CO}_2$ for a one hour period. The seed coat of the fruit was pierced by a surface sterilized stainless steel wire before (48,24 hrs), after (24, 48 hrs) or at the time of labeling (0 hr). The fruit were harvested 72 hrs after source leaf labeling, dissected, dried and processed for scintillation counting. Six branches were used per experiment, each of which was repeated 4 times. Histogram bars represent the percentage of total radioactivity in the plant tissue. Data was transformed by arcsine transformation for statistical analysis. Mean separation within columns by Tukey's studentized range test ($P < 0.05$).

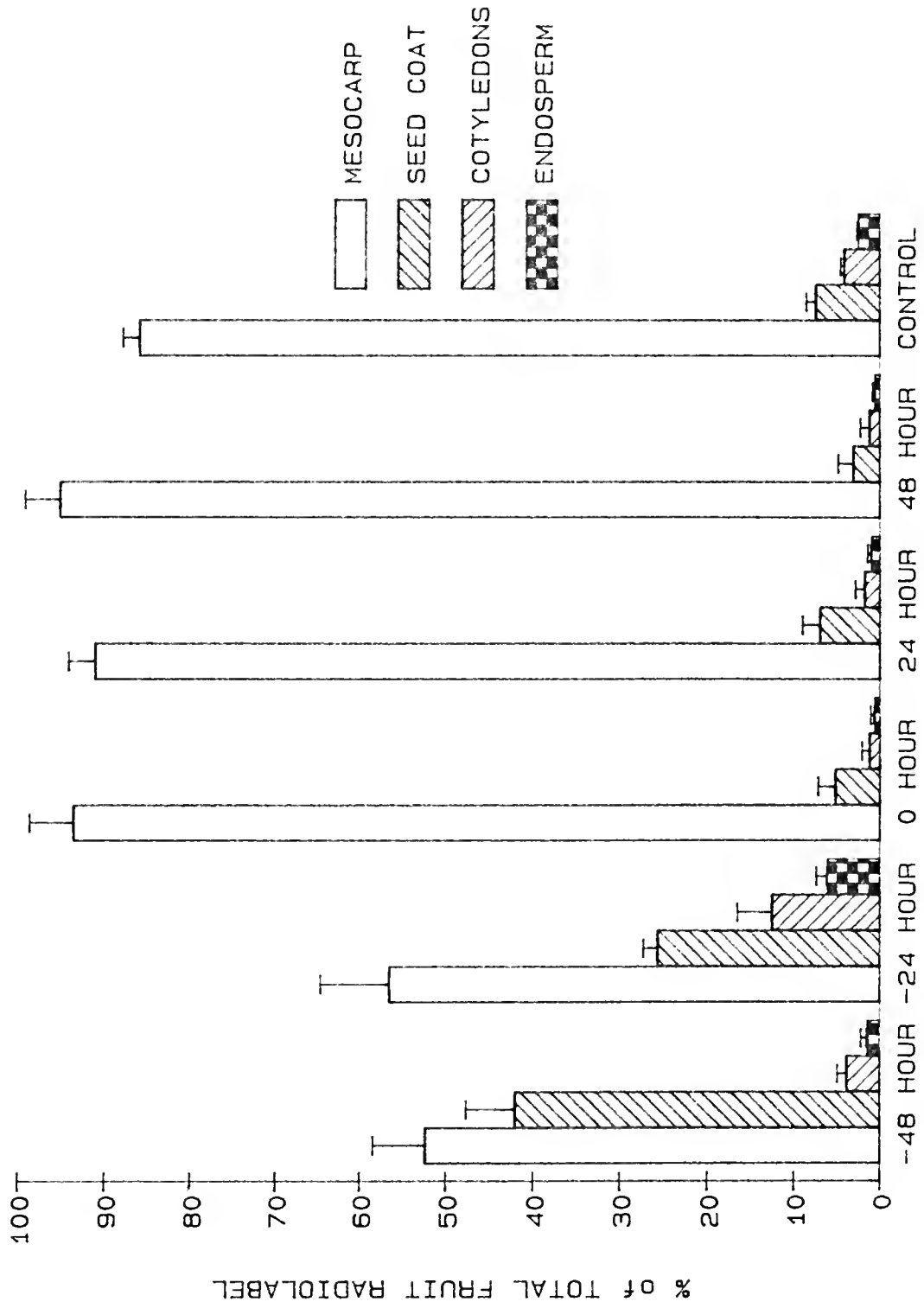
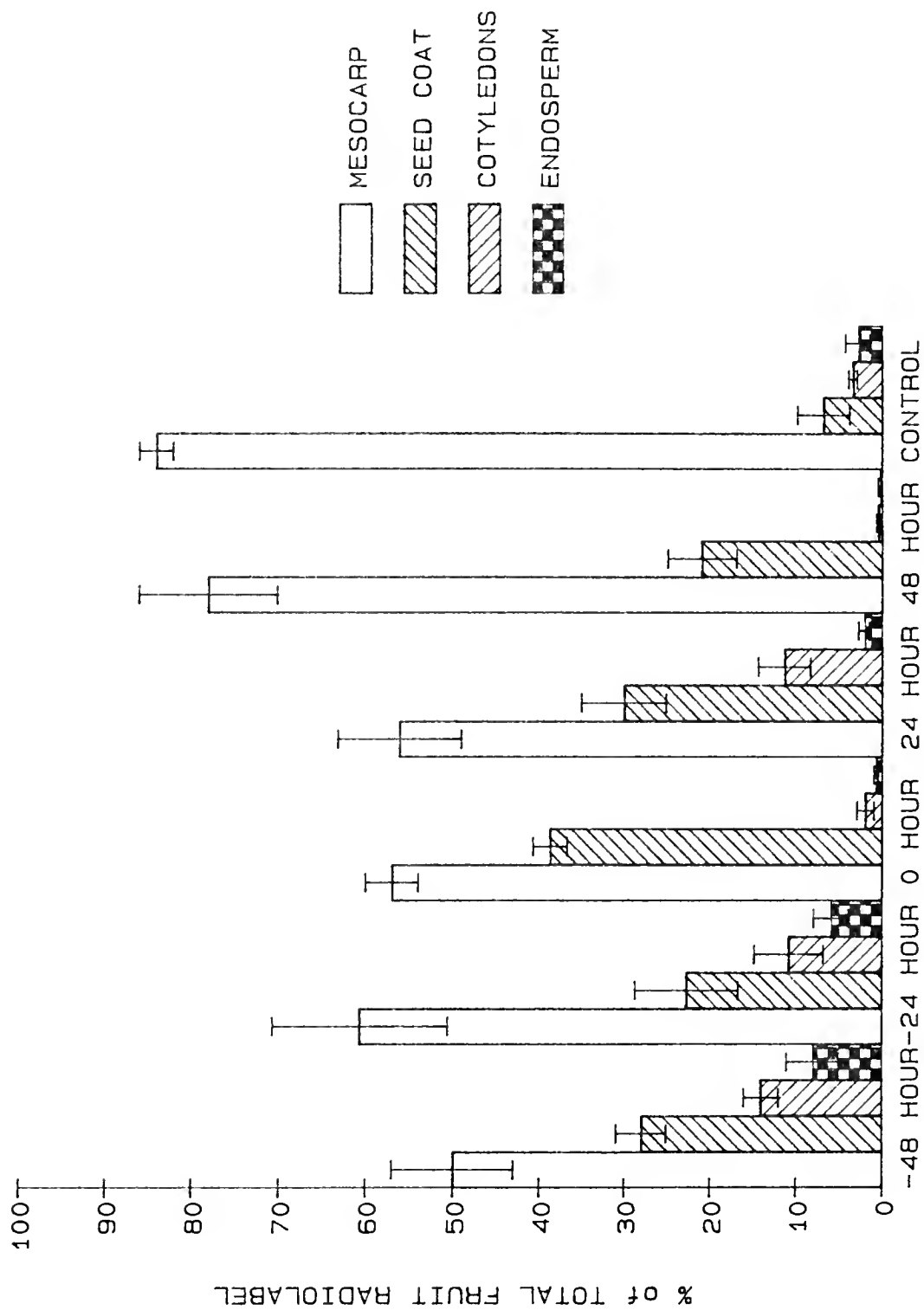


Figure 4-2. Distribution of ^{14}C in the Pierced Fruit of the Double-fruited Inflorescence. Source leaves on branches bearing two fruit were fed $^{14}\text{CO}_2$ for a one hour period. The seed coat of one fruit was pierced by a surface sterilized stainless steel wire before (48,24 hrs), after (24, 48 hrs) or at the time of labeling (0 hr). Both fruit were harvested 72 hrs after source leaf labeling. The fruit were dissected, dried and processed for scintillation counting. Six branches were used per experiment, each of which was repeated 4 times. Histogram bars represent the percentages of total radioactivity in the plant tissues. Data was transformed by arcsine transformation for statistical analysis. Mean separation within columns by Tukey's studentized range test ($P<0.05$).



CHAPTER 5 SINK/SOURCE RELATIONS IN THE AVOCADO INFLORESCENCE

Introduction

The photosynthetic contribution of reproductive organs to their own growth varies among species. The reproductive structures of barley (183), wheat (60,120,183), rice (58), oats (110) and other grasses (147) contribute between 50 and 75% of the needed photosynthate to the developing grain. In contrast, the reproductive organs of bean (40), soybean (153), cotton (57) and peas (66) are supplied assimilate primarily by the leaves. Although CO_2 fixation occurs in the reproductive organs of the latter group respiratory losses offset any net photosynthetic gain (153).

Significant differences in the fixation of carbon by the flowers and fruit of perennial tree crops have also been noted. Hansen (88) noted that substantial amounts of $^{14}\text{CO}_2$ were fixed by apple flowers prior to flower opening. Fixation decreased with petal drop and remained depressed until the fruit enlarged. Moreover, developing lemon (21) and orange fruit (13), and Valencia orange flowers (196) have been shown to contribute quantitatively little photosynthate to the developing fruit. Young avocado and mango fruit also fixed CO_2 (186), but the large respiratory loss in young fruit offset the net gain (4,186). Similar investigations on avocado flowers have not, to our knowledge, been done.

Avocado flowers have light, yellowish green petals. Their opening behavior has recently been reviewed by Davenport (46). The flowers are perfect, possessing both stamens and a single pistil. In the case of 'Petersen', a Type A cultivar (46), the flowers initially open in the morning as functionally female flowers. These flowers close at midday and then reopen the next day in the afternoon as functionally male flowers. Mature avocado trees produce up to 1.6 million flowers per tree, but only 0 to 0.66 percent of these flowers yield mature fruit (27). This large quantity of flowers may represent a substantial carbon expenditure; thus, the potential for assimilate competition among flowers and between flowers and fruits exists (20,156). A photosynthetic flower could benefit with regard to sink competition for stored or limited photosynthates. In companion reports, we describe the distribution of recent photoassimilates from source leaves into reproductive organs and other sinks of avocado. This report describes the distribution of those recent photoassimilates among and within buds, flowers and fruitlets.

Materials and Methods

Ten year old trees of Persea americana Mill. cv. 'Petersen' growing on 'Walden' rootstock at the Tropical Research and Education Center, Homestead, FL were used in this study. Tissues to be labeled were enclosed in a sealed plastic bag and fed with 20 μCi of $^{14}\text{CO}_2$ for a one hour period between 0900 and 1100 a.m. $^{14}\text{CO}_2$ was generated from $\text{NaH}^{14}\text{CO}_3$ (Amersham, specific activity 54 mCi mmol^{-1}) using equimolar amounts of 5% lactic acid.

Entire inflorescences or fruit were radiolabeled at several times during reproductive development. A schematic representation of an early stage of reproductive development is shown in Fig. 5-1. In this figure, leaves located distal to the inflorescences are at the center. Four individual, cymose inflorescences are shown inserted into the main axis of the stem. The older (proximal) leaves are located basipetal to the inflorescences. Individual inflorescences may include small (round <2mm), intermediate (round 2-3.5mm), elongated flower buds (elongate >3.5 mm), as well as open flowers, post-anthesis flowers (opened 4 or 24 hours earlier), and fruits. A total of 434 small flower buds, 350 medium flower buds, 270 elongated flower buds, 101 open flowers, 113 post-anthesis flowers and 25 fruitlets were evaluated in this study.

Flower Sink Strength

Single proximal leaves were labeled as previously described for the studies on reproductive structure sink strength. The branches composed of the distal leaves, inflorescences, and proximal leaves were removed and dissected into their component parts e.g., leaf, flower (corolla, stamens, pistil, nectaries and staminodes) 24 hours after labeling. The tissues were dried, weighed and digested with a commercially available tissue solubiliser (Fisher; Scintigest) as described by Burrell and Brunt (25).

CO₂ Fixation by Floral Structures

After removing leaves developing distal to the inflorescence, the flowering panicles were enclosed in plastic bags and fed with 20 uCi of ¹⁴CO₂ for a one hour period. Branches bearing inflorescences and

leaves proximal to the inflorescence were removed immediately after the labeling period. The branch tissues were then dissected into leaf, branch (distal and proximal to the inflorescence), and reproductive structures (panicals, receptacles, fruit, corolla, pistil, staminodes and nectaries) and prepared as described above for scintillation counting. Some branches were labeled in the morning when the open flowers were functionally female and other branches were labeled in the afternoon when the open flowers were functionally male.

Relative CO₂ Fixation

Branches composed of fruit- and flower-bearing inflorescences with leaves distal and proximal to the inflorescence were enclosed in plastic bags and fed with 20 uCi of $^{14}\text{CO}_2$ for a one hour period. The branches were harvested immediately after labeling and then dissected into reproductive structures (fruit, flowers, buds, receptacles and pedicels), new leaves (developing distal to the fruit), and old leaves (present on the proximal or basipetal side of the fruiting structures). These tissues were processed as described above for scintillation counting. In this paper flowers which had opened previously were referred to as post-anthesis flowers.

The average lamina length of the leaves distal to the inflorescence was less than 20 mm. The leaves distal to the inflorescence at this stage of development were sinks and not sources of assimilate (Chap. 7).

Carbon Fixation by Fruit

Individual fruit (diameter 4 cm) were enclosed in sealed plastic containers and then labeled with 100 uCi of $^{14}\text{CO}_2$ as previously

described. Fruit were harvested at 24 and 48 hrs after labeling. The fruit were separated into the peel (exocarp), mesocarp, seed coat, cotyledons and endosperm and processed for scintillation counting as described above.

The relative specific activity (RSA) as defined by Mor and Halevy (81,139) reflected the changes in the growth pattern of sinks. The RSA ($\%dpm \ \%dry \ mass^{-1}$) was calculated as the distribution of label or current photoassimilate content relative to the distribution of dry mass. Deviations from unity reflected changes in the allocation of assimilate and hence changes in growth patterns. Values greater than unity indicate that proportionately more assimilate is being utilized by the organ in question in relation to the existing tissue. Sink intensity is defined as the relative distribution of radiolabel on a $\%dpm \ mg^{-1}$ basis or alternatively as the $dpm \ mg^{-1}$ (139). Sink strength is defined as the the percentage of the total label recovered from the tissues.

Results

Proximal Leaf Labeling

A schematic representation of an early stage of reproductive development is shown in Fig. 5-1. Typically at this stage of reproductive development, each panicle would be composed of several inflorescences bearing flower buds, flower buds and flowers or flower buds, flowers and fruit. The insert of Fig. 5-1 is an example of the last panicle type since it bears flower buds, open flowers, post-anthesis flowers, and fruit.

The data from proximal leaf labeling experiments are given in Table 5-1. The RSA expresses the relationship between the partitioning of current photosynthate and the matter previously allocated to that particular organ. Any deviation of the RSA from unity indicates a change in the growth pattern of the tissue. The competitive ability of a tissue can also be expressed by the sink intensity. Small and intermediate buds incorporated almost twice as much of the translocated labeled assimilate (dpm mg^{-1}) as did the elongated buds (Table 5-1). Similarly, the sink intensity and RSA of small ($8.1 \text{ \%dpm mg}^{-1}$, $2.20 \text{ \%dpm \%mg}^{-1}$) and intermediate buds ($9.4 \text{ \%dpm mg}^{-1}$, $2.6 \text{ \%dpm \%mg}^{-1}$) were very similar to each other but were almost 2-times that of the elongated buds ($4.5 \text{ \%dpm mg}^{-1}$, $1.23 \text{ \%dpm \%mg}^{-1}$). In fact, intermediate buds were growing more rapidly as measured by the RSA ($2.6 \text{ \%dpm \%mg}^{-1}$) than the majority of the tissues examined. Flower bud growth as measured by sink intensity and RSA increased until the elongated bud stage when both sink intensity and RSA decreased dramatically consistent with impending flower opening.

Partitioning of the translocated labeled assimilates occurred within the flower (Table 5-1). Although all of the floral tissues actively incorporated radiolabeled assimilates, the nectaries of the open flowers exhibited the greatest sink intensity ($11.6 \text{ \%dpm mg}^{-1}$) and RSA ($3.17 \text{ \%dpm \%mg}^{-1}$). The partitioning of labeled assimilates in the post-anthesis flower differed from that observed in open flowers. The corolla exhibited the greatest sink intensity and RSA in post-anthesis flowers. The sink strength, sink intensity and RSA of the pistil,

stamens and nectaries all decreased post-anthesis which may reflect the higher rate of flower abscission occurring at this point or the lack of growth in these mature tissues.

The fruit had the greatest sink strength but the lowest sink intensity and RSA. Fruit incorporated 35.2% of the total radioactivity recovered from the inflorescence yet the fruit comprised 71.5% of the total dry mass. Sink strength in this case was more a reflection of mass than of sink intensity.

CO₂ Fixation by Reproductive Structures

The relative distribution of dry mass and fixed ¹⁴CO₂ within a flower was the same at both the male and female stages (Table 5-2). The corolla and nectaries comprised approximately 60% of the flowers dry mass. The stamens and pistil comprised approximately 26% and 13%, respectively, of the flower dry mass. Greater than 85% of the total flower radioactivity was recovered in the corolla.

Differences were observed in the mass and specific activity of flowers at different stages of development (Table 5-3). The mass of flowers open at the female stage (.00450 gm) was significantly less than that of flowers open during the male stage (.00509 gm). The specific activity of the open male flower was greater than the open female flower (data not shown). The flower buds fixed more CO₂ than the open or post-anthesis flowers.

The mass of post-anthesis flowers was also significantly greater at the male stage (.0052 gm) than the female stage (.0039 gm). During the male stage, open flower and post-anthesis flowers had similar mass (0.0050 gm vs. 0.0052 gm) while open flowers and post-anthesis flowers

during the female stage had very different dry mass (0.0045 vs. 0.0039). Because of the dicogamous flowering habit of avocado, the age of the post-anthesis flowers was different in the male and female stages. Flowers that were post-anthesis during the female stage had first opened 24 or 48 hours earlier. Flowers that were post-anthesis at the male stage could have opened 4 or 24 hours earlier. The lower dry mass of the older post-anthesis flowers of the female stage suggested that as the flower matured or senesced the dry mass decreased.

Experiments were done to compare the relative $^{14}\text{CO}_2$ fixation by various components of a branch (Table 5-4). Results shown in Table 5-4 are typical of 5 replicate branch labelings done in this series.

The branch described here had a main axis and four subterminal branches bearing new flushes of leaves with average midvein lengths less than 20 mm. In addition to flower buds and flowers there were 21 fruits which ranged in diameter from 1 to 11 mm. The older, proximal leaves fixed the majority (86.3%) of $^{14}\text{CO}_2$ recovered from the branch. The new leaves fixed only about 10% of the total $^{14}\text{CO}_2$ recovered from the branch. However, they were 50% as efficient as the mature leaves in fixing $^{14}\text{CO}_2$ on a dry mass basis.

Fruits and flowers fixed very small amounts of $^{14}\text{CO}_2$. The total $^{14}\text{CO}_2$ fixed (1.63%) and the specific activity (1.2 dpm mg^{-1}) of the flowers were greater than that of the fruits ($.57\%$, $.98 \text{ dpm mg}^{-1}$, respectively). Fixation of $^{14}\text{CO}_2$ by the fruits increased with the diameter and paralleled the increase in dry mass (Fig. 5-2). The

majority (>90%) of label remained in the peel and mesocarp (Table 5-5) of the labeled fruit. Between 1 and 3% of the label was recovered in tissues other than the fruit.

The majority (>90%) of the $^{14}\text{CO}_2$ fixed by the fruit remained in the exocarp or peel (Table 5-5). A significant amount of label was recovered in the mesocarp. Little label was recovered in the seed coat, cotyledons or endosperm. No differences were observed in assimilate distribution in fruit harvested 24 or 48 hours after labeling. The limited translocation of fruit-fixed assimilates was not surprising, since avocado fruit undergo continuous cell division and expansion throughout development (167). Although the mesocarp is highly vascularised any available assimilate would be rapidly metabolized by the growing cells. A small amount of radioactivity was recovered from other tissues (leaves, apex, wood). Minor, but significant translocation from the fruit has been noted in apple (7,86) and strawberry (6).

Discussion

Source/sink relations in avocado during reproductive growth has been an area of active research in this laboratory, particularly with regard to source-limitation-induced abscission. The avocado is a good model for this type of study, since multiple sinks represented by concurrently developing flowers, fruit and leaves, compete for hypothetically limiting amounts of photoassimilates. Work in this laboratory has shown that locally produced photoassimilates are not limiting reproductive growth and that source/sink relations in avocado

change with leaf flush age (Chap.7,8). The leaves located proximal to the inflorescence supply assimilate to the developing leaves and fruit and to the roots. As the leaves developing distal to the inflorescence mature, assimilates from the leaves proximal to the inflorescence no longer move acropetally but move primarily towards the roots. When matured, leaves immediately distal to the inflorescence supply assimilate to the fruit, another developing flush of leaves and to the roots. When the final flush of leaves has matured all of the translocated assimilates move basipetally toward the fruit and roots.

The study of source/sink relations would not be complete without examining how assimilates were distributed within the reproductive organs and if the reproductive organs could provide enough assimilates for their own growth. The photosynthetic capabilities of the reproductive organs of plants vary drastically (148,183). Although most of these studies have been done in agronomic crops, tree crops appear to exhibit the same diversity. Citrus flowers contribute very little photosynthate to their growth (13,186,196), while apple flowers (88) contribute significant photoassimilate to their growth. Although the flowers' role as a source of photoassimilate varies with the plant, its status as a weak sink is well established (199).

The RSA (%dpm %dry mass⁻¹), sink intensity (%dpm mg dry mass⁻¹ and sink strength (%dpm) values for flower buds all peaked when the buds were of intermediate size. The sink intensity (8.1 %dpm mg dry mass⁻¹ and RSA (2.2 %dpm % dry mass⁻¹) of small buds was also relatively high compared to the more developed elongated buds (4.5 %dpm mg dry mass⁻¹, 1.23 %dpm % dry mass⁻¹). The most actively assimilating tissues were the younger less developed flower buds. As the floral structures

developed (elongated bud stage) the RSA, sink intensity and sink strength decreased. Similar data were reported in apple (88). Apple flower buds were most active in fixing CO_2 and in accumulating translocated label in floral stage c (flowers are open in stage e). High sink activities have also been noted prior to anthesis in soybean (23). In soybean this high sink intensity is associated with the maturation of the embryo sac.

The tissues of the open flower were supplied assimilates by the leaves proximal to the inflorescence (Table 5-1). Partitioning of assimilate to the nectaries was accentuated, as indicated by the high RSA ($3.17 \text{ \%dpm \%dry mass}^{-1}$), during the 24 hour period before the flower initially opened. The increase in label in the nectaries probably resulted from the accumulation of sugars in the nectar. Tissues of flowers that were post-anthesis at harvest were weaker sinks than open flowers i.e., flowers post-anthesis accumulated less label than open flowers. Flower abscission at this time was very high. The one exception was the corolla of the post-anthesis flowers which had the greatest increase in the assimilation of radiolabel (RSA = $3.6 \text{ \%dpm \%dry mass}^{-1}$) of any tissue examined. The increased sink intensity of the corolla post-anthesis may have reflected the initiation of senescence events. During the 24 hour period post-anthesis the petals and sepals of the corolla elongated and changed in color from a light green to a pale yellow. Davenport (45) has shown that ethylene production by avocado flowers increased nearly 20-fold within 24 hours post-anthesis (male stage). Similar climacteric-like rises in ethylene have been observed in rose petals (63) and carnation (142,143). The increase in ethylene production is followed by increased membrane

permeability, wilting and death (22,63,64,142,143) or abscission (81,178). The induction of enzyme systems involved in flower senescence may account for the increased sink intensity of the corolla; however, remobilization of metabolites within the flower, particularly from the nectaries (119) and the stamens (103), may have contributed to the accumulated label recovered in the corolla.

Avocado flowers are light green in color and exhibited some photosynthetic capabilities. Male flowers fixed 28% more $^{14}\text{CO}_2$ on a dry mass and a per flower basis than did female flowers (Table 5-3). Flowers labeled during the male or female stage exhibited similar mass and radiolabel distributions. The pistils of male and female flowers fixed similar percentages of the total amount of $^{14}\text{CO}_2$ fixed and comprised similar percentages of the total dry mass (Table 5-2) of the flower. The dry mass and $^{14}\text{CO}_2$ content of the corolla (including nectaries) and the stamens varied slightly with the sexual stage of the flower. Male flowers had a greater mass and dpm percentage in their stamens than did open female flowers (Table 5-3).

Post-anthesis flowers were more massive during the male stage than the female stage although their photosynthetic capacity had decreased (Table 5-3). The initiation of senescence events after anthesis may account for the progressive decrease in photosynthetic abilities of post-anthesis flowers. The decreased mass of post-anthesis flowers (female stage) may be attributed to respirational mass loss or to advanced senescence events since these flowers are 24 to 48 hours older than male-stage post-anthesis flowers. However, the cause for the lower mass of open flowers during the female stage remains unexplained.

The fruits exhibited the lowest RSA ($.49 \text{ \%dpm \%mg dry mass}^{-1}$) and sink intensity ($1.8 \text{ \%dpm dry mass}^{-1}$) of any reproductive tissue (Table 5-1), yet because of their mass they had the greatest sink strength (35.2 \%dpm). Brun (23) attributed the low sink intensity of post-anthesis fertilized soybean flowers to the availability of cytoplasmic starch grains. The reason for the low sink intensity in very small fruits of avocado is unknown; however, small avocado fruit have a very high respiratory rate (4,186) and CO_2 derived from translocated assimilates may be lost in respiration.

Respiratory losses in small avocado fruit (4,186) offset photosynthetic gains (186). Respiration and carbon fixation (Fig.5-2) correlated to the mass of the avocado fruit (186). In citrus, mass and respiration correlated only in young fruit (186). Todd et al. (186) suggested that the bimodal respiration curves of citrus corresponded to the transition from cell division to cell expansion. Since avocado fruit undergo continuous cell division (168) throughout development this bimodality would not be expected.

Avocado fruit fixed less than 4% of the radiolabel available to a branch (Table 5-4). Carbon fixation was correlated to fruit mass and diameter (Fig. 5-2); other studies using detached fruit (186) found that photosynthesis per unit area remained constant in growing avocados. Although avocado fruit photosynthesis clearly does not play a major role in fruit growth, it may be involved in some regulatory processes. Avocado fruit preparations contain RuBisCo and PEPCase (33). PEPCase activity has been noted in several plants (54,196) during early fruit development, particularly in fruit whose respiratory

losses exceeded photosynthetic gains. In citrus, the organic acids produced by PEPCase were used directly as respiratory substrates in the TCA cycle (21). Photosynthesis in avocado fruits may have a similar role.

In conclusion, all of the reproductive structures of the avocado inflorescence were capable of fixing $^{14}\text{CO}_2$. However, assimilates translocated from other organs, such as the proximal leaves, provided the majority of necessary photosynthate for growth. The avocado flower contributed very little photosynthate to its own development. However, the flowers and flower buds were also relatively weak sinks so the significance of flower carbon fixation to growth and respiration has not been determined. Avocado flowers exhibited a stage-dependent mass difference; flowers in the male stage were significantly more massive than flowers in the female stage. Fixation of carbon by the fruit correlated to fruit dry mass. Very little translocation of fruit-fixed assimilates was noted. Although all of the structures of the avocado inflorescence fixed CO_2 , their contribution to the carbon budget appeared to be minor.

Table 5-1. Distribution of Radioactivity Supplied by Proximal Source Leaves to the Avocado Inflorescence.

The following data represent a composite of at least 20 inflorescences. Sink intensity is defined as the relative distribution of total radioactivity on a %dpm mg dry mass⁻¹ basis. Dry mass is given in grams. Percentage ¹⁴C or the sink strength is the percentage of the total ¹⁴C recovered from the reproductive tissues. The relative specific activity (RSA) is defined as the % dpm % dry mass⁻¹.

Tissue	Dpm	Dry Mass	dpm/mg	% ¹⁴ C	Sink Intensity	RSA
Pedicle	3025840	0.4068	7438	18.90	10.3	2.9
Buds						
Very Small	949771	0.1636	5805	5.90	8.1	2.2
Small	2155190	0.3185	6767	13.50	9.4	2.6
Medium	945325	0.2927	3230	5.90	4.5	0.8
Bud Scales	130580	0.0514	2540	0.8	3.5	1.0
Flowers						
Open						
Pistil	109078	0.0282	3868	0.7	5.5	1.4
Stamens	576404	0.0822	7012	3.60	9.7	2.8
Nectary	612686	0.0730	8393	3.80	11.6	3.2
Corolla	914801	0.1778	5145	5.70	7.1	1.9
Mature						
Pistil	40799	0.0172	2372	0.3	3.3	0.8
Stamen	226814	0.0417	5439	1.40	7.5	2.0
Nectary	116555	0.0298	3911	0.7	5.4	1.5
Corolla	571739	0.0660	8863	3.60	12.3	3.6
Fruit	5630112	4.3966	1281	35.20	1.8	0.5

Table 5-2. Fixation of $^{14}\text{CO}_2$ by Open Flowers.

Open flowers were labeled² during both the male and female stages. The flowers were harvested, dissected and processed for scintillation counting immediately after labelling. The nectaries were included with the corolla.

Tissue	Male Flowers		Female Flowers	
	% Dry Mass	% dpm	% Dry Mass	% dpm
Pistil	13.6 \pm 2.4	4.2 \pm 1.5	12.6 \pm 1.8	4.5 \pm 0.3
Stamens	29.1 \pm 0.2	9.5 \pm 0.3	23.0 \pm 2.5	5.9 \pm 0.8
Corolla	57.2 \pm 2.2	86.3 \pm 1.3	64.4 \pm 2.0	89.6 \pm 0.5

Table 5-3. Relative Specific Activity of Inflorescence Components.

Labeled inflorescences were harvested immediately after labeling and divided into the groups for processing. At the 95% confidence level the dry mass of male and female flowers are significantly different.

Male Flower Stage

Tissue	Mean Dry Mass	% dpm/mg	% dpm/Flower
Whole Open Flower	.0051 \pm .00037	17.7	26.7
Mature Flower	.0052 \pm .00059	5.7	8.5
Small Bud <3mm	.0022 \pm .00016	40.5	27.2
Medium Bud >3mm	.0035 \pm .00029	36.1	37.5

Female Flower Stage

Tissue	Mean Dry Mass	% dpm/mg	% dpm/Flower
Whole Open Flower	.0042 \pm .00006	12.6	19.2
Mature Flower	.0039 \pm .00001	10.8	13.7
Small Bud <3mm	.0019 \pm .00001	45.0	32.3
Medium Bud >3mm	.0031 \pm .00013	31.6	34.8

Table 5-4. Early Season Branch Labeling.

Entire branches were labeled for 1 hour and then immediately harvested. The branches were dissected and processed for scintillation counting.

Tissue	#	Midvein Length (mm)	dpm	% ¹⁴ C (% of total)	% ¹⁴ C (%dpm/mg)
Old Proximal Leaves	5	70.9 + 14	2,737,446	86.3	63
New Distal Leaves	31	18.8 ± 4	320,466	10.1	33
Fruit	21		18,072	0.57	0.98
Fruit Receptacles			6,024	0.19	0.92
Fruit Peduncles			38,046	1.2	1.4
Miscellaneous (flowers, buds, panicals)			51,680	1.63	1.2

Table 5-5. Radiolabel Distribution after Photosynthetic $^{14}\text{CO}_2$ Assimilation by Fruit.

Fruit were labeled for 1 hour in the light with $^{14}\text{CO}_2$ and then harvested 24 or 48 hrs later. Fruit were peeled, dissected and tissues were processed as described in the Materials and Methods. A total of 4 and 9 fruit were harvested after 24 and 48 hr chase periods, respectively, with a mean radiolabel of $278,616 \pm 32,168$ and $205,546 \pm 13,446$ dpm, respectively.

Tissue	dpm % of total recovered	Dry Mass (% of total)	dpm % of total recovered	Dry Mass (% of total)
Exocarp	52.2 ± 10.3	21.4 ± 1.6	56.8 ± 7.2	22.3 ± 4.3
Mesocarp	44.9 ± 7.5	65.5 ± 2.3	41.9 ± 7.2	66.6 ± 3.9
Seed Coat	0.7 ± 0.2	9.2 ± 0.4	0.7 ± 0.2	7.1 ± 0.4
Endosperm	0.12 ± 0.03	1.1 ± 0.1	0.15 ± 0.03	1.1 ± 0.1
Cotyledon	0.1 ± 0.05	2.9 ± 1.1	0.15 ± 0.04	3.0 ± 0.9

Figure 5-1. Sink Strength of Floral Components. The schematic diagram represents the avocado inflorescence in relation to the new distal leaves and the older proximal leaves. The insert is a detailed representation of a pedicel and its component parts. Sink strength is defined as the relative content of radiolabel on a $\% \text{dpm mg dry mass}^{-1}$ basis.

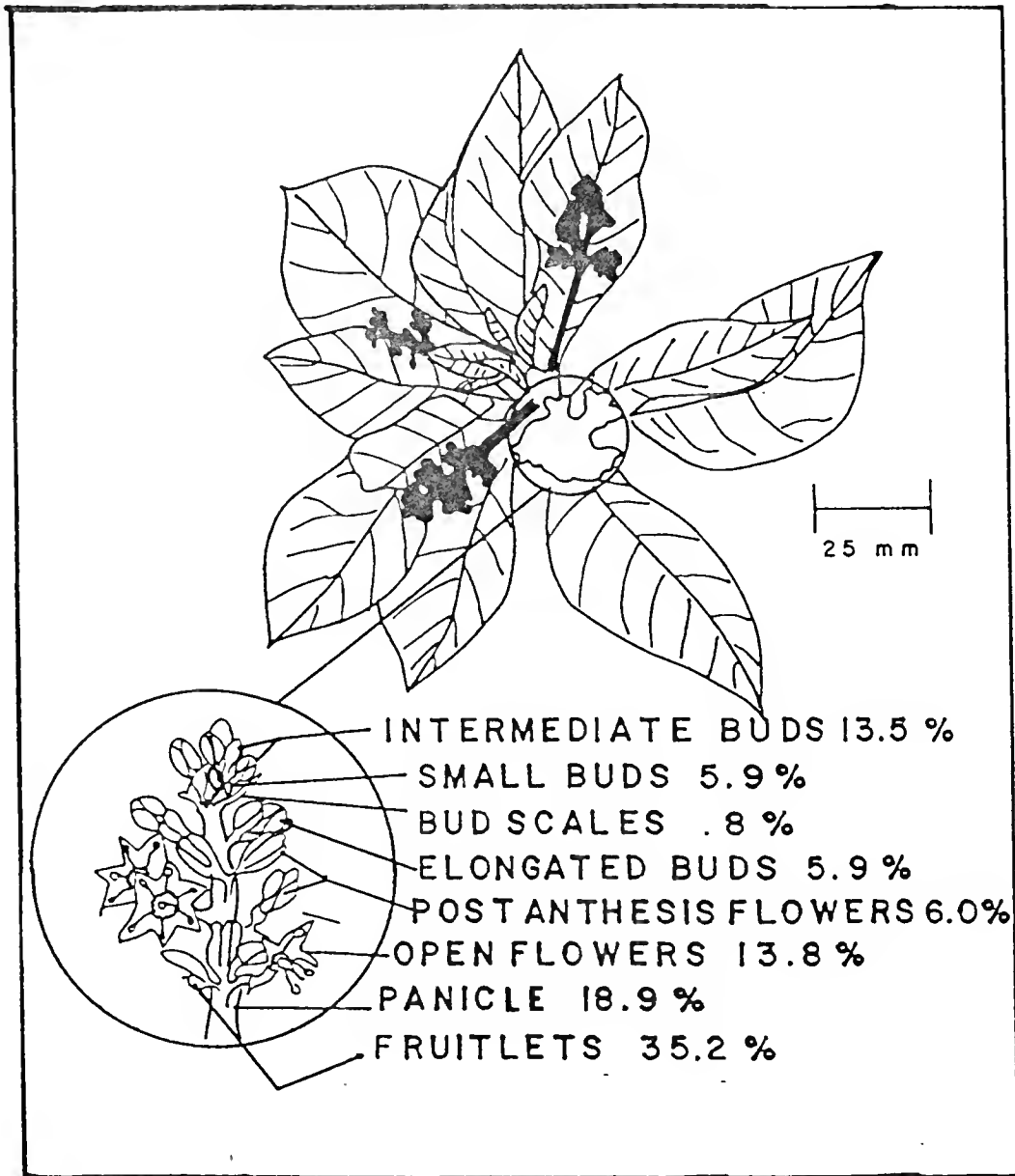
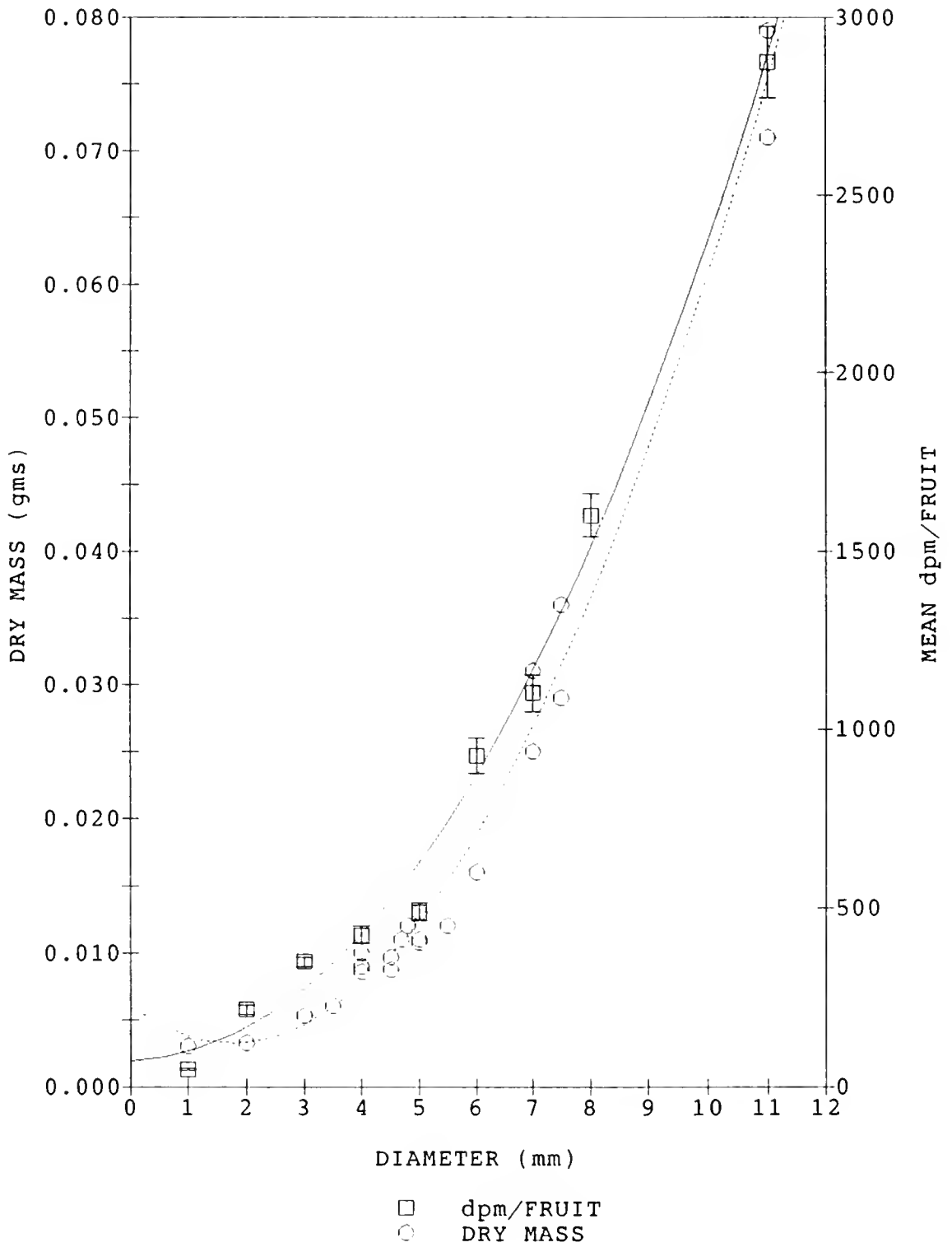


Figure 5-2. Early Season Labeling of Avocado Fruit. Entire branches were labeled. The branches were dissected and processed for scintillation counting immediately after the labeling period. Dry mass and average dpm of radiolabel fixed per fruit is plotted against fruit diameter (mm).



CHAPTER 6
DISTRIBUTION OF RECENT PHOTOASSIMILATES IN AVOCADO:
THE ROLE OF PROXIMAL LEAVES

Introduction

Carbohydrate reserves have been shown to play an integral role in early season growth in deciduous trees (88,155) where the carbon supply comes solely from those reserves. In evergreen trees, however, both current photosynthates and stored reserves contribute metabolic resources to developing sink tissues (121). The cyclical pattern of starch accumulation in the wood of avocado is well documented (26,162) and has led to the hypothesis that low levels of starch reserves induce fruitlet abscission and alternate bearing syndrome. Existing mature leaves in evergreen trees also serve as sources of both reserve carbohydrates and current photoassimilates. Their role in early season growth of flowers and fruitlets has not been evaluated.

Depending on the cultivar, avocado flower initiation begins in late November to January in Florida (44,158). By mid-January the terminal buds destined to become inflorescences have enlarged and are morphologically distinct from vegetative and dormant axillary buds. The development of the inflorescence has been described elsewhere (44,46,158,164).

The development of the inflorescence is concurrent with resumption of vegetative growth by the terminal bud (158,164,197). Concurrent growth and development of the inflorescence, fruitlets, and vegetative termini coupled with the high flower and fruitlet abscission rates

noted during this period (44) has fostered the assumption that the developing floral and vegetative tissues compete for limiting amounts of assimilates (162). It follows from the commonly observed, high abscission rates of reproductive organs that fruitlets are less competitive than the developing distal leaves or other major sinks, i.e., branches and roots, in mobilizing assimilates. The purpose of this study was to examine the contribution of leaves, located proximal to the inflorescence, to the development of the distal tissues and to determine if the leaves distal to the inflorescence compete with the developing reproductive organs for recent photoassimilates.

Materials and Methods

Ten-year-old trees of Persea americana Mill. cv. 'Peterson', growing in Rockdale soil at the Tropical Research and Education Center, Homestead, FL were used in this study. Branches (1-2 cm diameter) were girdled 2 to 3ft from the tip 24 hours prior to each labeling experiment. A single, intact leaf, located proximal to the inflorescence on each experimental branch, was enclosed in a 2-liter, plastic bottle and fed 20 uCi of $^{14}\text{CO}_2$ during an hour period between 0900 and 1100 hr $\text{NaH}^{14}\text{CO}_3$ (Amersham with a specific activity of 54 mCi/mmol) was injected through a serum vial stopper into the bottle. An equimolar amount of 5% lactic acid was injected into the bottle to generate $^{14}\text{CO}_2$. Leaves were labeled on various dates during inflorescence, and fruitlet development, and distal bud expansion (January through June).

Translocation of assimilates was monitored by measuring the decrease in radioactivity in leaf punches taken from the labeled source leaf. Two punches, totaling .618 cm², were sampled from the leaf immediately after labeling and again 48 hr later. Treatment branches, including all of the leaves, flowers, and fruitlets located distal to the girdle were harvested 48 hours after labeling. Two leaf punch samples were taken from each of the mature, proximal leaves (approximately 10 per branch). Distal tissues were sampled in their entirety. Cross-sectional slices (2-3 mm wide) of each branch (wood, bark) were sampled at locations both basipetal and acropetal to the labeled leaf. All samples were oven dried at 56°C until reaching constant mass, weighed, digested with a commercial tissue solubiliser (Scintigest - Fisher Scientific) and decolorized with 30% H₂O₂ as described by Burrell and Brunt (25) prior to liquid scintillation counting.

Forty-five separate labeling experiments were done over a period of two years. The results are the means of at least ten replicate samples taken at four different developmental stages described in Results.

Results

The results of the labeling experiments were grouped into four stages based on the average midvein length of the distal leaves; stage 1 the average midvein length of the distal leaves was <20 mm, stage 2 the average midvein length of the distal leaves was between 20 and 30 mm, stage 3 the average midvein length was between 30 and 50 mm and stage 4 the average midvein length was greater than 50 mm. During the

early stages of flush development (distal midvein length <20 mm, Fig. 6-1) the inflorescence was still expanding. The active sink tissues were the developing flower buds and distal leaves. Labeled assimilates from the source leaf moved in both the acropetal and basipetal directions. No label was recovered from the mature non-source leaves proximal or distal to the source leaf. Forty-three percent of the recently fixed carbon remained in the source leaf 48 hours after labeling (Fig. 6-1). Of the 29% of label recovered in the tissues distal to source leaf, 15.39% was recovered in the leaves and 13.61% was recovered in the inflorescence and developing fruitlet tissues. The amount of ^{14}C -assimilates recovered in each tissue was proportional to the dry mass of the tissue (Table 6-1). For example, during the earliest stage (midvein length <20 mm), reproductive mass represented 52.9% of the total harvested dry mass and accounted for 53.06% of the total ^{14}C assimilated by the harvested tissues.

The average midvein length of the distal leaves was 20 to 30 mm at the next stage of development (Fig. 6-2). All stages of flower bud development were present on the inflorescence. Translocation of photoassimilates from the source leaf was occurring at the same rate as in the previous stage i.e., 57-59% of the labeled carbon was translocated from the source leaf in 48 hours; however, the proportion of label traveling basipetally was much lower. Only 10.7% of the radiolabel was traveling basipetally while 48.2% of the label was recovered in the distal tissues. The distribution of label between the reproductive and vegetative tissues again was directly related to the

dry mass distribution. At this stage the inflorescence had enlarged, accounting for most of the dry mass increase in the reproductive tissues.

During the period when midvein lengths were 30 to 50 mm (Fig. 6-3), changes in assimilate movement from the proximal source leaves suggested the sink to source transition (8,29) of the distal leaves was occurring. Total export of radiolabel from the source leaf decreased from 59% to 48%. The proportion of label traveling to the distal tissues also decreased by 27%. Reproductive tissue dry mass represented 75% of the expanding biomass.

When the average distal midvein length was greater than 50 mm the distal leaves were no longer sinks for assimilate from the proximal leaves. Very little radiolabel (.145% of the translocated label) was recovered in the distal tissues; however, the proportion of label in fruitlet and leaf tissues again paralleled the dry mass ratios (Fig. 6-4). Fruitlets at this stage represented 51.6% of the total mass (Table 6-1) and contained 47.4% of the label translocated acropetally.

This sink-to-source conversion was paralleled by a decrease in export from the older proximal leaves (Fig. 6-4). In the early stages of flush development (Figs. 6-1 and 6-2), the proximal leaves exported greater than 55% of their recently fixed photoassimilates in 48 hours. When the average midvein length was between 30-50 mm the export of assimilates from the proximal source leaves decreased to below 50%. The amount of label going to the distal tissues also decreased. In the final stage of sink to source transition, when the average midvein

length was 50 mm or greater, the proximal leaf exported only 31.4% of its recently fixed photoassimilates. Almost all of this label traveled basipetally.

Discussion

The concept of competition between individual fruitlets and leaves as an explanation for premature fruit abscission is well entrenched in the literature (35,36,76,138,162,191,197). Carbohydrates play an integral role in this explanation since fluctuation in plant starch content at times appears to parallel fruit yield. In fact treatments which alter starch content, such as girdling (35,36,107,124,132,138, 191, 197) often increase yields. These types of data are frequently used to support the notion that carbohydrate limitation induces abscission.

Scholefield et al. (162) noted seasonal fluctuations in starch content of avocado. They suggested that low yields were the result of competition between the developing vegetative terminus and the fruitlets. Their data indicate, however, that the peak in starch content coincides with the peak in fruit and flower abscission. In this case starch, availability did not affect abscission. Conflicting data have been presented on the importance of the starch content in biennial bearing citrus trees. Some workers (76) have described significant correlations between the starch content and the bearing status of trees, while others (112,130) found no clear correlation between carbohydrate levels and tree productivity. The evidence in support of stored carbohydrates as the tangible link to fruit abscission is strictly correlative.

Furthermore increasing starch levels is not always effective in increasing yields. Girdling increases the starch content of the leaves and wood above the girdle yet in some varieties of avocado girdling does not increase yields (36). Stutte and Martin (181) increased the starch content of olive trees 5-fold yet they were unable to change the alternate bearing pattern. Interestingly, killing the olive seed embryo overcame the alternate bearing problem without affecting fruit dry mass accumulation. Similar results were obtained in apple (78,79) and orange (130) which further negates the suggestion that starch levels directly affect fruitlet dry mass accumulation or fruitlet abscission.

Although carbohydrates are absolutely essential for the growth of fruitlets and new leaves, work in this laboratory has shown that carbohydrate supply from nearby leaves is not a factor in fruitlet abscission. Mature leaves proximal to the inflorescence were more than capable of providing assimilate for new growth. In fact, mature leaves proximal to the inflorescence translocated assimilate both acropetally towards the developing apex and basipetally towards the trunk. The basipetal sink for assimilates from the proximal leaves in avocado differed from the basipetal sink for assimilates observed in mango (32). Mature mango leaves proximal to the labeled source leaf accumulate translocated labeled assimilates; however, mature leaves of avocado did not act as sinks for translocated assimilates.

The paucity of label translocated to the distal leaves of stage 4 inflorescences (Fig. 6-4) from the proximal source leaf reflected the maturation and increased autonomy of the distal leaves which supply the developing fruits. Similar results were obtained in strawberry (160),

apple (17,109) and orange (121) where mature leaves failed to import radiolabeled assimilates. The distal leaves do in fact become sources of assimilate for the fruitlets when their midvein length is between 35-45 mm (Chap. 7). Upon maturation of the leaves distal to the avocado inflorescence, even more assimilate was available; however, fruitlet abscission continued long after maturation of the distal leaves as has been described elsewhere (Chap. 2).

Fruitlet and developing leaves did not compete for recent photoassimilates. The amount of radiolabel assimilated by a tissue was directly proportional to the dry mass of the tissue i.e., the mobilizing ability of floral and vegetative tissue was the same at the level of the whole fruitlet and flower (Table 1). Floral and vegetative sinks in avocado have also been shown to have similar sink strengths for nitrogen (208). Floral tissue dry mass and leaf dry mass were about the same during the first stage (midvein length <20 mm). During the next two stages fruit mass was 2-3 times greater than leaf mass. Although the developing floral tissues were growing rapidly, no difference was seen in the specific activities of the two different sinks. This suggests that neither tissue was limited for assimilates and that both tissues were accumulating assimilates at their maximum rate.

In conclusion, the accumulation of recent photoassimilates by the developing floral and vegetative tissues was mass dependent. During the early stages of reproductive flush development carbohydrate availability appeared to be sufficient to support the growth of leaves and fruitlets and did not limit fruitlet growth or stimulate fruitlet abscission.

Table 6-1. Radiolabel Distribution to the Distal Tissues from the Labeled Source Leaves Located Proximal to the Avocado Inflorescence.

Source leaves, proximal to the inflorescence, were fed $^{14}\text{CO}_2$ for a one hour period. The entire branch was harvested 48 hours later. The branch was dissected, dried and processed for scintillation counting. The reproductive tissues include the bud scales, flower buds, open flowers, senescent flowers, receptacles and peduncles. Mass is given in grams.

Mean Midvein Length	Dry Mass (g)		14C-Assimilate Localization (dpm)			
	Reproductive Structures	Leaf Tissues	Reproductive Structures	Leaf Tissues	Total Export	Total export to Distal Tissue
<20	0.2441	0.114	3,126,317	2,761,286	11,590,578	5,887,036
<30	0.4069	0.1801	5,068,949	2,553,531	15,814,274	7,622,480
<50	1.0539	0.5546	11,251,902	4,753,648	36,369,420	16,005,551
>50	1.5547	1.4564	11,026	123,206	52,534,912	234,232
(% of total dpm recovered)						
<20	52.9	47.1	53.1	46.9	56.5	28.7
<30	66.4	33.6	66.5	33.5	58.9	48.2
<50	67.3	32.7	70.3	29.7	48.4	21.3
>50	51.8	48.4	47.4	52.6	31.4	0.15

Figure 6-1. Distribution within the Branch of ^{14}C -Assimilates from the Proximal Source Leaf when the mean Midvein Length of the Distal Leaves was less than 20 mm. Source leaves were fed $^{14}\text{CO}_2$ for a one hour period. The entire branch was harvested 48 hours later. The branch was dissected, dried and processed for scintillation counting. The schematic drawing indicates the approximate developmental stage of the reproductive flush from mid-March to early April. The black spots indicate the labeled source leaf. The percentages are based on the total radiolabel recovered after the initial 1 hr $^{14}\text{CO}_2$ fixation period. The arrows indicate the direction of assimilate flow. The data represent the mean values of at least 10 replicates. Standard error of measurement was 5% or less.

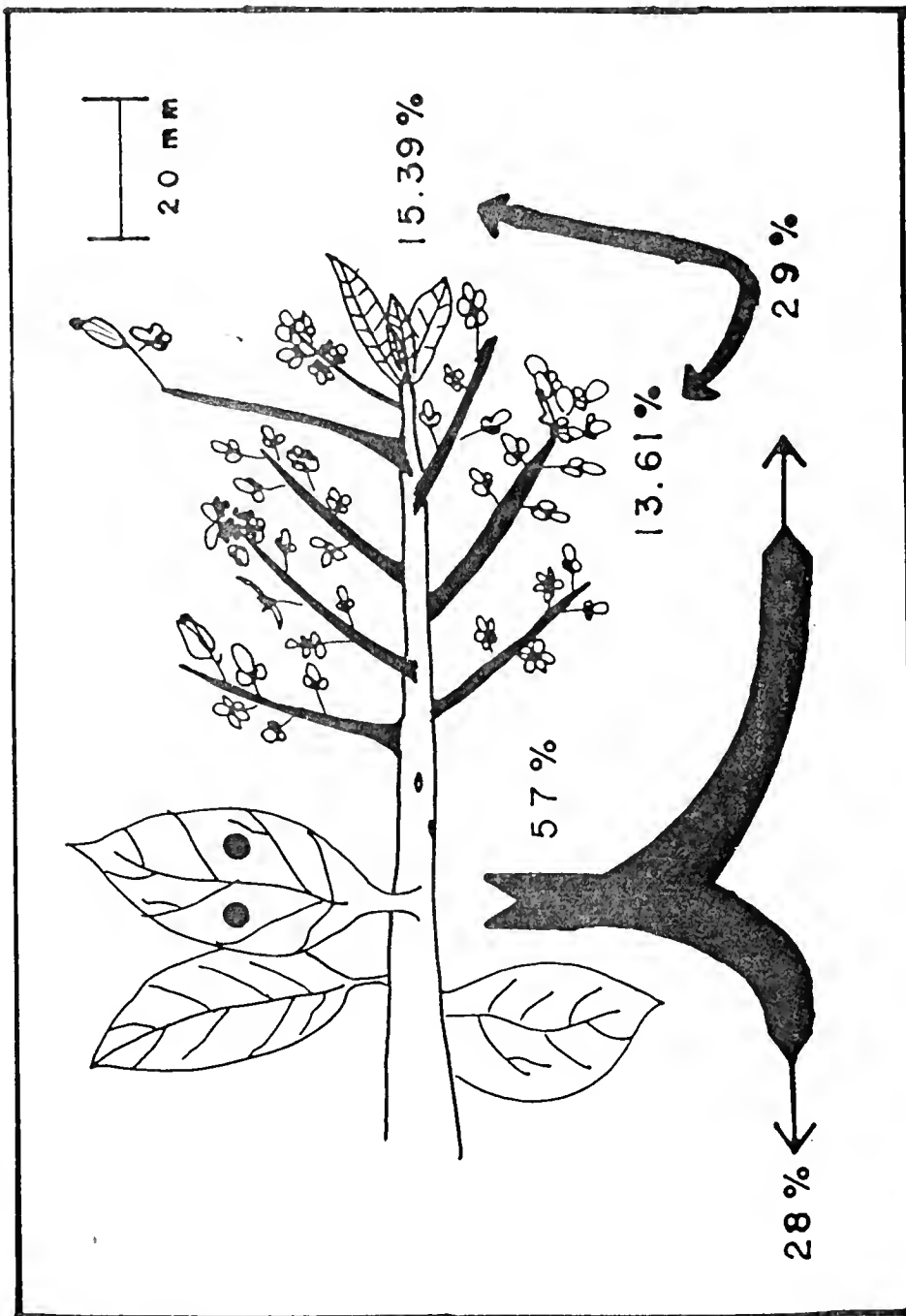


Figure 6-2. Distribution within the Branch of ^{14}C -Assimilates from the Proximal Source Leaf when the mean Midvein Length of the Distal Leaves was between 20-30 mm. Source leaves were fed $^{14}\text{CO}_2$ for a one hour period. The entire branch was harvested 48 hours later. The branch was dissected, dried and processed for scintillation counting. The schematic drawing indicates the approximate developmental stage of the reproductive flush from early to mid-April. The black spots indicate the labeled source leaf. The percentages are based on the total radiolabel recovered after the initial 1 hr $^{14}\text{CO}_2$ fixation period. The arrows indicate the direction of assimilate flow. The data represent the mean values of at least 10 replicates. Standard error of measurement was 5% or less.

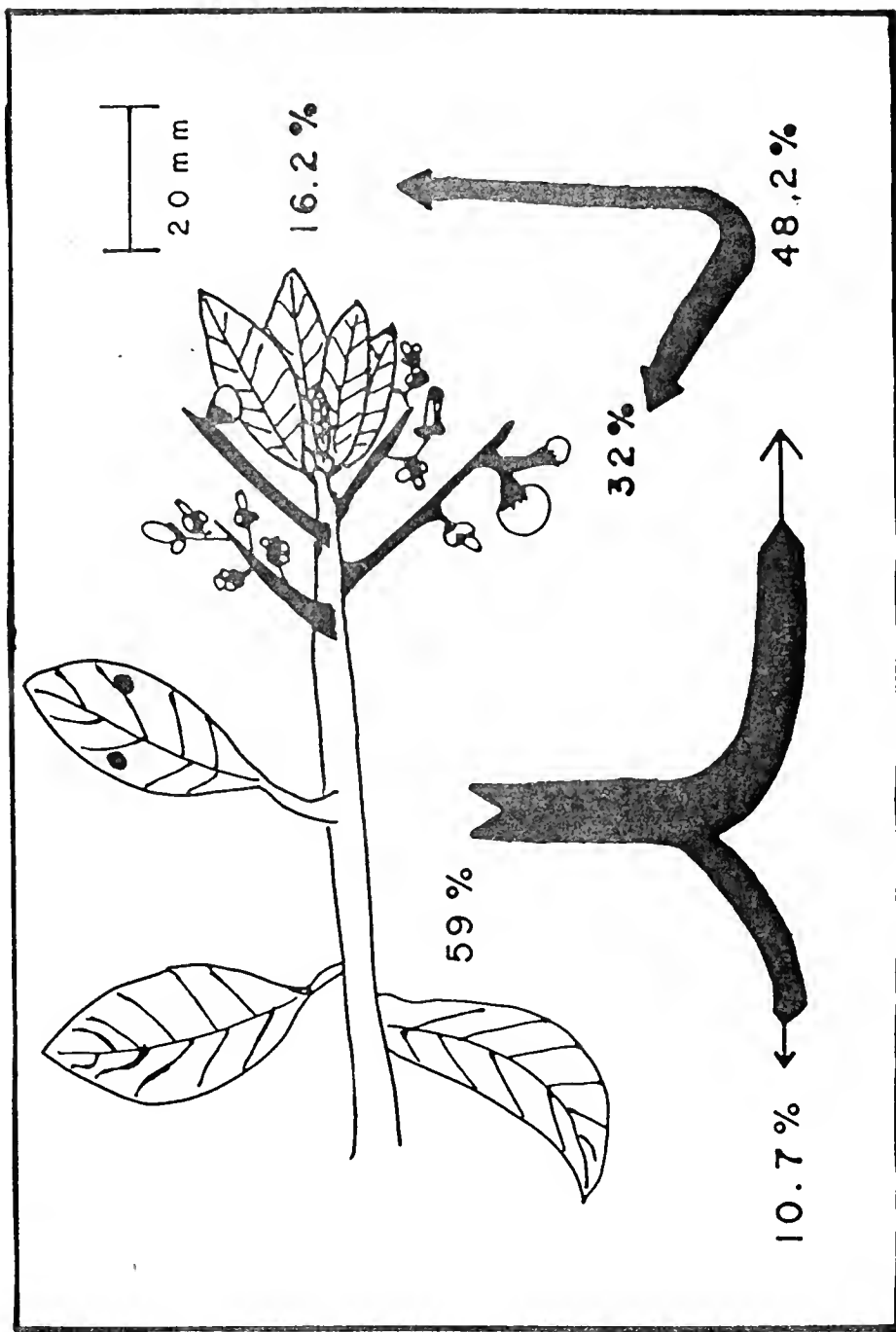


Figure 6-3. Distribution within the Branch of ^{14}C -Assimilates from the Proximal Source Leaf when the mean Midvein Length of the Distal Leaves was between 30-50 mm. Source leaves were fed $^{14}\text{CO}_2$ for a one hour period. The entire branch was harvested 48 hours later. The branch was dissected, dried and processed for scintillation counting. The schematic drawing indicates the approximate developmental stage of the reproductive flush from mid-April to early May. The black spots indicate the labeled source leaf. The percentages are based on the total radiolabel recovered after the initial 1 hr $^{14}\text{CO}_2$ fixation period. The arrows indicate the direction of assimilate flow. The data represent the mean values of at least 10 replicates. Standard error of measurement was 5% or less.

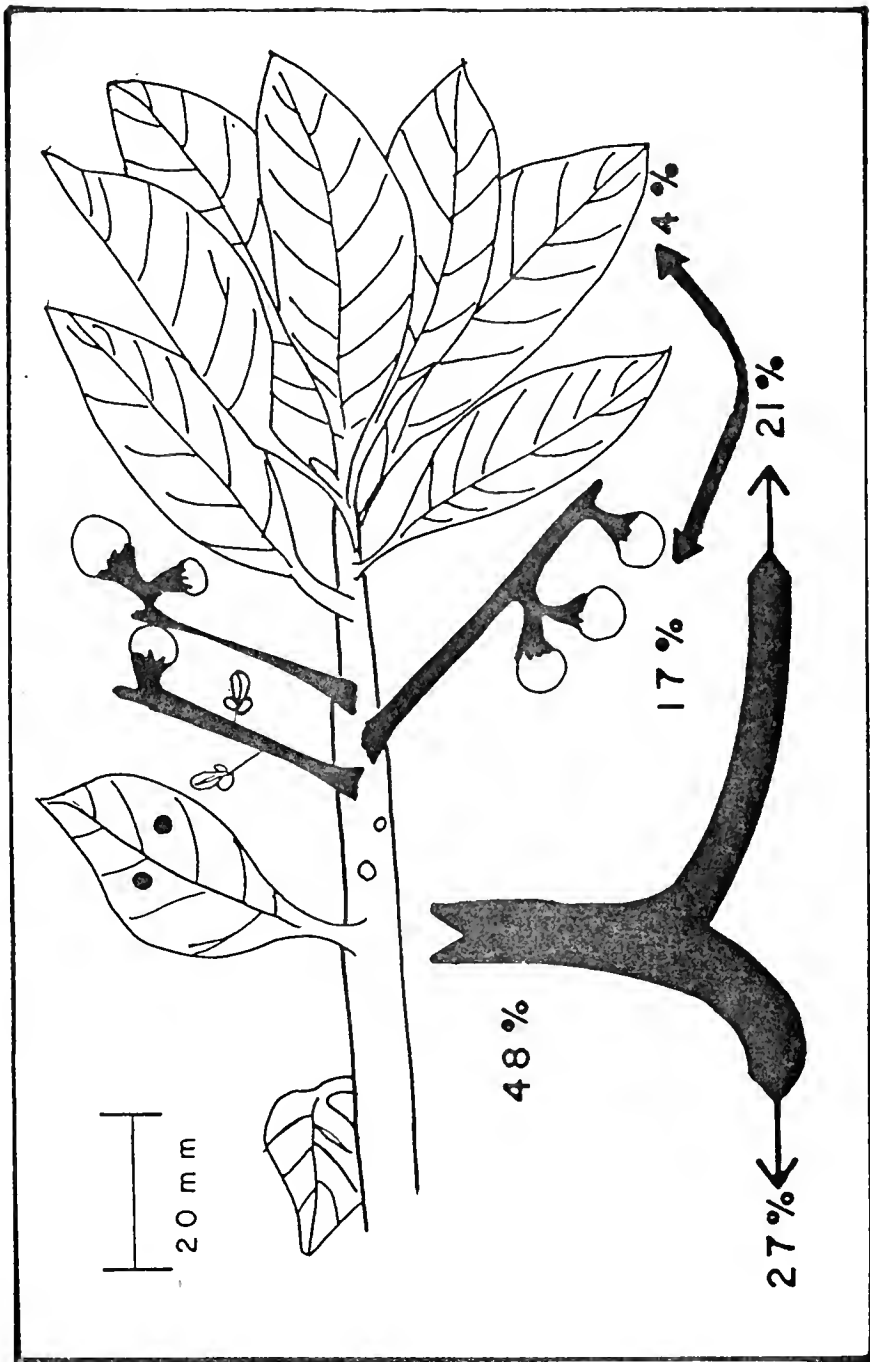
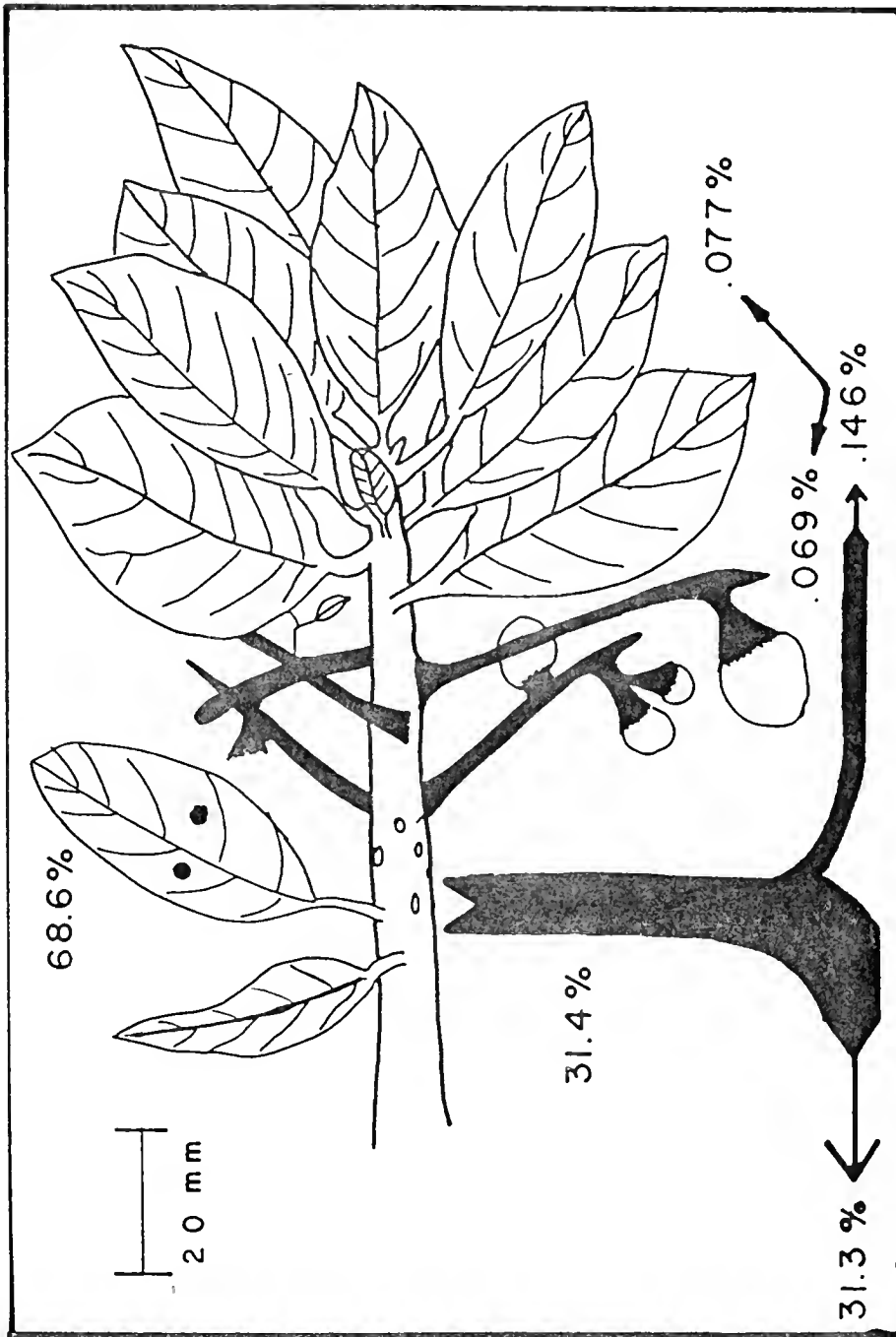


Figure 6-4. Distribution within the Branch of ^{14}C -Assimilates from the Proximal Source Leaf when the mean Midvein Length of the Distal Leaves was greater than 50 mm. Source leaves were fed $^{14}\text{CO}_2$ for a one hour period. The entire branch was harvested 48 hours later. The branch was dissected, dried and processed for scintillation counting. The schematic drawing indicates the approximate developmental stage of the reproductive flush from early to mid-May. The black spots indicate the Labeled source leaf. The percentages are based on the total radiolabel recovered after the initial 1hr $^{14}\text{CO}_2$ fixation period. The arrows indicate the direction of assimilate² flow. The data represent the mean values of at least 10 replicates. Standard error of measurement was 5% or less.



CHAPTER 7
SINK TO SOURCE TRANSITION OF DEVELOPING LEAVES LOCATED DISTAL
TO AVOCADO INFLORESCENCES

Introduction

Developing leaves are known to be net importers (sinks) and mature leaves net exporters (sources) of photoassimilated carbon. Recent photoassimilates from mature leaves of tomato were reported to translocate to the developing leaves at rates according to the age of the developing leaves and the leaves' phyllotactic relationship to the source leaf (96). The conversion of leaves from sinks to sources involves both physiological and structural changes. These include an increase in net photosynthesis (51,73,95,101), a decrease in assimilate import (101,192,193,203), the onset of phloem loading (65,192) and alterations in leaf anatomy (108).

The development of leaf autonomy in avocado is of particular interest, since immature leaves developing distal to the inflorescence represent a potentially 'competing' sink with developing flowers and fruits for available assimilate. The inflorescences of avocado emerge subterminally (44). New leaves form at the apex, distal to the inflorescence, shortly after the onset of flowering and develop concurrently with flowers and early setting fruit. The concurrent growth of the reproductive organs and new leaves coupled with the high rate of fruitlet abscission (44) observed during this period has led some workers (20) to suggest that these developing sinks compete for a limited amount of available assimilate. Furthermore, reducing the

vegetative sink strength of the apex by shoot tipping i.e., removing the developing leaves distal to the inflorescence, allowed the fruits to establish their mobilizing ability and increased yield (20).

The hypothesis that fruit abscission is induced by the limited availability of carbohydrates is based on two assumptions. First, that the leaves proximal to the inflorescence cannot provide adequate assimilate for the concurrent growth of fruits and new leaves. Second, that the distal leaves are immature and thus importers not exporters of assimilate during this period of development and fruit abscission. However, work done in this laboratory indicated that older leaves located proximal to the inflorescence (proximal leaves) supplied enough assimilate to support the development of both the new leaves and fruits (Chap 6). Furthermore, the growth of the vegetative bud is temporary; abscission occurs during flowering and early fruit set and continues well after the termination of distal vegetative growth.

The development of the new distal leaves and their contribution to fruit growth has not been examined. The purposes of this research were two-fold. First, to determine when the distal leaves become exporters of assimilate. Second, to characterize the movement of assimilates from mature distal source leaves to developing fruit.

Materials and Methods

Ten-year-old, field-grown trees of Persea americana Mill. cv. 'Petersen' were used in this study. Leaves to be labeled were enclosed in a sealed plastic bag and labeled with 20 uCi of $^{14}\text{CO}_2$ for a one hour

period between 0900 and 1100 hr. $^{14}\text{CO}_2$ was generated from $\text{NaH}^{14}\text{CO}_3$ (Amersham, specific activity of 54 mCi/mmol) using equimolar amounts of 5% lactic acid.

Development of the distal flush was examined by labeling individual leaves located proximal to the inflorescence (assimilate import studies). Branches were harvested at 24 or 48 hrs. and processed as described below.

Leaf and branch samples were dried at 56°C for three days. The samples were weighed, digested with a commercial tissue solubiliser (Scintigest - Fisher) and decolorised with 30% H_2O_2 (25). When the samples were decolorized, 20 mL of scintillation fluid was added to the samples for counting.

The entire distal leaf mass (assimilate export studies, net photosynthetic capacity studies) or individual distal leaves (assimilate export studies, phyllotaxy studies) were also labeled. Entire branches were harvested at 0, 24 or 48 hours after labeling and dissected immediately.

The leaves were removed from the branches after noting their relative orthostichy. The length of the midvein (MVL) was measured. Leaves with MVL less than 30 mm were processed in their entirety for scintillation counting. Leaf samples ($.618\text{ cm}^2$ discs) were taken from leaves with $\text{MVL} > 30\text{ mm}$ and processed for scintillation counting.

Positions of flowers and fruit were also noted before removal from the branch. Reproductive tissues were used in their entirety and processed for scintillation counting.

Net photosynthesis was calculated from leaf punch samples ($.618\text{ cm}^2$) removed from the source leaf immediately after the one hour pulse of $^{14}\text{CO}_2$. The amount of translocated assimilate was calculated by

subtracting the amount of radiolabel remaining in the source leaf at 24 hr. post-labeling from the amount of label in the source leaf at 0 hr. The relative rate of export was defined as the percentage of net radioactive photosynthate translocated from the source leaf. The relative rate of import was defined as the percentage of the total radioactive translocate imported by the sink leaf.

The distribution of radiolabel in the leaves distal to the inflorescence was indicated as the percentage of the total radioactivity recovered from the distal leaves. Similarly, the distribution of label in fruit at a particular orthostichy was calculated as a percentage of the radioactivity recovered from all of the fruit.

Results

Import/Export

The relative rate of import or the percentage of the translocated assimilate imported by distal leaves as they developed is shown in Fig. 7-1. Small leaves imported little of the total translocated assimilate. As the leaf enlarged to 20% of the final midvein length (FMVL) the relative import ratio peaked. As the leaf continued to enlarge the import of assimilates by the developing distal leaf decreased and was negligible when the leaf reached 37% FMVL.

The relative rate of export (Fig. 7-1) was measured by the difference in label present at 0 and 24 hours post-labeling in labeled source leaves located distal to the inflorescence. Export began at 20% final midvein length and reached a plateau at 60% final midvein length. The amount of radiolabeled assimilates translocated in 24 hours was

similar to that of mature proximal leaves. The peak in assimilate import and the onset of assimilate export occurred during the same stage of leaf maturation.

Whole Branch Labeling

The relationship between leaf age, midvein length and the leaf specific activity is shown in Fig. 7-2. The entire flush of distal leaves was labeled and the branch was harvested either immediately after labeling or 24 hours post-labeling. The specific activity of the leaves increased gradually with increasing midvein length until the midvein length approached 30 mm. The specific activity of leaves with midvein lengths between 30 and 40 mm increased 7 fold. However, translocation of assimilates from the distal leaves to the fruit had apparently not begun since radiolabel was not recovered in the inflorescence or proximal to the inflorescence.

Proximal Leaf Labeling

Individual proximal leaves were labeled at various stages of distal leaf development. The amount of label going into each orthostichy of distal growth was determined. At the earliest stage of distal leaf development (average midvein length <20 mm), labeled assimilates from the proximal leaves were equally distributed among all orthostichies (Fig. 7-3). When the proximal leaves were labeled at the next stage of development (average midvein length between 20-30 mm), the distribution of radiolabeled assimilates was skewed (Fig. 7-4), with the greatest amount of label (42%) going to the leaves in alignment with the source leaf. A total of 73% of the radiolabel in the new leaves was present in leaves in direct alignment with or adjacent to the source leaf position. When the average midvein length of the

distal leaves was between 30 and 40 mm (Fig. 7-5), 98% of the label was present in the leaves in direct alignment or adjacent to the source leaf position. Leaves in direct alignment accumulated 50% of the label recovered in leaf tissue.

Distal Leaf Labeling

The partitioning of translocated assimilates from the mature distal leaves was examined by labeling individual distal leaves and then determining the distribution of radioactivity at each orthostichy. When there were no fruits present on the branch with the same orthostichy as the source leaf, the distribution of the translocated assimilate was skewed to favor a single adjacent orthostichy (Fig. 7-6). However, when fruits were present which had the same orthostichy as the labeled source leaf, these fruits received greater than 94% of the label recovered in fruit tissues (Fig. 7-7). The remainder of the radiolabeled assimilates was dispersed to the fruits at other orthostichies.

Effect of Distance and Fruit Mass on Assimilate Distribution

When several fruits on separate peduncles had the same phyllotaxy as the labeled source leaf, then fruit size and parastichy determined assimilate distribution (Fig. 7-8). For example, fruits (5,8) which have the same phyllotaxy as the source leaf contained only 52% of the radiolabeled assimilate while fruits (3,7) at the adjacent orthostichy contained 45% of the radiolabel. In this particular case, the fruits at the adjacent position were nearer the source leaf and their mass was twice that of the other fruit.

Mature Distal Leaves

Individual mature leaves distal to the inflorescence were labeled and the rate of translocation and the sites of label distribution were observed. Within 48 hrs, 69% of the initially fixed carbon was translocated from the labeled source leaf. The fruit and reproductive structures accumulated 29% of the initially fixed carbon. No radiolabel was recovered from the leaves proximal to the inflorescence although the wood of the branch proximal to the inflorescence contained radioactivity.

Discussion

Other work from this laboratory has examined the source/sink relations of the flowers, fruit and leaves proximal to the inflorescence (Chap. 6). From these studies, it was learned that the leaves proximal to the inflorescence were the major sources of recent photoassimilate for the developing vegetative and reproductive tissues. However, the amount of assimilate moving acropetally to these sinks modulated with the developmental stage of the leaves distal to the inflorescence. The decrease in acropetal assimilate movement suggested that the sink to source conversion of the leaves occurred when the average midvein length of the leaves in the flush was between 30 and 40 mm. An in-depth study of the sink to source conversion of the distal leaves was not done in these earlier works.

Import of assimilate by developing avocado leaves increased dramatically between 10 and 20% final midvein length. The rate of import then declined until it was almost negligible at 37% final

midvein length. The increasing rate of assimilate import reflected the increase in sink leaf size. The subsequent decline of assimilate import reflected the decrease in areas of the leaf still importing assimilate. The increase and subsequent decrease of assimilate import noted in developing leaves of avocado has also been observed in the leaves of cucumber (101), squash (203), beet (65) and soybean (185) during their sink to source transitions.

The export of label from avocado leaves was first observed when the leaves were 20% final midvein length. Avocado leaves began export of assimilates at a younger developmental stage (20% final midvein length) than most other plants (65,172,187,203), with the exception of tomato leaves (97) which initiated export at 10% final lamina length. Cucumber (95) and beet (72) leaves initiate export of assimilates at 30% and 25% final lamina length, respectively. Early studies linked the initiation of export to the cessation of cell expansion (42); however, others (65) have recently suggested that initiation of export depends on the osmotic pressure or the soluble sugar content of the sieve elements and companion cells.

The increase in the export rate of developing avocado leaves was very gradual. The gradual increase in export capacity however may have been a reflection of the sampling technique combined with the leaf maturation and not a true measurement of export from the leaf. Samples were taken from the lateral vein sections between the midvein and the growing edge. Since leaves mature basipetally (127) the central midvein sections may export assimilate to other less mature areas within the leaf. If so, this shift of label within the leaf would

appear as a gradual increase in export out of the leaf. Similar results were obtained in beet where phloem loading as determined by autoradiography (65) began at 25% final midvein length; however, export from the leaf was not observed until 35 percentage final midvein length.

Export from avocado leaves reached a plateau at a slightly later developmental age (60% final midvein length) than other plants (65,72,117,203). In these other studies export commenced between one third to one half leaf expansion and reached a plateau at approximately 50% final lamina length. When avocado leaves were 65% final midvein length the rate of export reached a plateau of $56 \pm 12\%$ of the daily fixed carbon. This value remained constant during continued leaf expansion. The rate of export or the daily export of carbon varies with the plant. Mature cucumber leaves export approximately 80% of their daily fixed carbon (95) and tomato leaves export around 70% (94), while beet leaves translocate only 25% (65) of their daily fixed carbon.

Between 20 and 40% final midvein length, the leaf was both exporting and importing assimilates (Fig. 7-1). The bi-directional movement of assimilates has been noted elsewhere (16,111,189) and generally occurs in leaves and petioles that are approximately half-grown (65,189). The period of export/import overlap is generally very brief. During this time sink, source and transition regions exist in a single leaf.

Net carbon fixation was noted in avocado leaves as small as 6.6% final midvein length (Fig. 7-2). Some workers have found net losses of carbon in very small leaves in the light (42), while others have found

net gains in leaves as small as 22% final midvein length (51). Net carbon fixation in avocado was constant on an area basis until the leaves reached 23% final midvein length when a dramatic increase in photosynthesis occurred. At this stage leaf import of assimilates was at its peak and export of assimilates had just begun.

Net photosynthesis has been reported to decline between 50-75% final midvein length (65,101) in beet and cucumber. Hopkinson (101) suggested that this may result from self-shading or other age related phenomena such as increased photorespiration. Net photosynthesis did not decrease in mature leaves during this study perhaps because unlike cucumber and beet, avocado is a perennial evergreen. The age of a 'Petersen' avocado leaf may be measured in years rather than weeks. Furthermore, canopy shading may not have a significant effect on leaf photosynthesis since avocado leaves saturate at low light intensities (163).

The pattern of radiolabel distribution in developing leaves depended upon sink leaf age and sink and source leaf phyllotaxis. Avocado exhibits a 2/5 phyllotaxis. As the leaves distal to the inflorescence matured, the effect of phyllotaxy on label distribution became pronounced. During the early stages of distal leaf development (MVL <20 mm) equivalent amounts of label were recovered from leaves at each orthostichy (Fig. 7-3). When the average distal lamina length was between 20 and 30 mm (Fig. 7-4), two adjacent orthostichies, one of which was in alignment with the source leaf contained the majority of radiolabel. The final stage, when the average distal lamina length was

between 30 and 40 mm (Fig. 7-5), showed an even more pronounced skewing of label assimilation with two adjacent orthostichies containing 98% of the label recovered from the leaves.

The transition from an equal to phyllotactically determined distribution of radiolabel reflected the maturation of the vascular system. The continuous, acropetal, helical differentiation of the procambium and phloem is well established (59,149,150) as is the helical movement of assimilates through the stem via the phloem (109). Leaves forming at the apex receive assimilates from the undifferentiated procambium (150). The differentiation of the vascular system with increasing plastochron age was evident in the final two developmental classifications where the leaves in direct alignment and at the next orthostichic position contained the majority of the radiolabel. Leaves with lamina lengths 30 mm or less contained the most label. Leaves larger than this were in transition from being sinks to sources or had become exporters of assimilate.

The path of assimilates exported from the mature leaves of avocado (Figs. 7-6, 7-7), as in other plants (85,94,113,117,121,126,176), was strongly guided by phyllotaxy. If avocado fruit were in phyllotactic alignment with the source leaf then these fruit contained 94.5% of the label recovered in the fruit (Fig. 7-6). If there were no fruit with the same orthostichy as the source leaf then the majority of the label (62.6%) recovered in the fruit was found in fruit located at the orthostichy 72° from the source leaf position (Fig. 7-7). Tangential movement of assimilates between orthostichies has also been shown to occur in beet (113), willow (151), bean (185), citrus (121) and tobacco (176).

In cases where there were no fruit in alignment with the labeled source leaf, label distribution reflected the tangential movement of assimilates and was moderated by the fruit mass and linear distance from the source leaf.

Phyllotaxy alone does not determine assimilate distribution in avocado. When several fruits on separate pedicels had the same phyllotaxy as the labeled source leaf, then size and parastichy determined assimilate distribution (Fig. 7-8). If the fruits were of similar size, then the fruits nearest the source accumulated slightly more labeled assimilate. If the fruit nearest the source was significantly larger, then it was the major sink for labeled assimilates; however, if the fruit farthest from the sink was larger then a more equal distribution of radiolabel was observed. The effect of size on assimilate distribution was related to the absolute growth potential of the fruit at that particular stage (94).

The supply of recent photoassimilates did not appear to limit fruit or leaf growth, or to partition to one fruit at the expense of another. Studies of assimilate movement indicated that 69% of the label was exported out of the source leaves in 48 hours (Fig. 7-9). Only 29% of this label was recovered in fruit tissues. No label was recovered in the mature leaves proximal to the fruit or in non-labeled leaves distal to the fruit. Label was recovered in the stem sections proximal to the fruit. Although the fruit are strong sinks (Chap. 5) less than half of the available translocated assimilates from the mature leaves distal to the inflorescence went to the fruit. Being the nearest sink to the source of assimilate, the fruit would also exert the greatest "pull" on

the translocated assimilates. However, the movement of labeled assimilates to the fruit and basipetally to the roots suggests that assimilates were ample and did not limit fruit growth.

In summary, leaves distal to the avocado inflorescence reached their peak of assimilate import when they were approximately 22% final midvein length. Export from these leaves commenced at this stage and reached a plateau when the leaves were approximately 60% final midvein length. At maturity, the distal leaves translocated 69% of their recently photoassimilated carbon in 48 hours. Radiolabel recovered in the fruit accounted for 29% of the assimilate, while the remainder of the label traveled basipetally. Assimilate movement from source leaves was strongly guided by phyllotaxy, although fruit size did influence label accumulation.

The apparent precocious maturation of the developing distal leaves suggested that the leaves were autonomous and hence, the period of competition between fruits and developing leaves for translocated assimilates was shorter than previously expected.

Figure 7-1. Relationship of Import and Export to Final Midvein Length. The relative import rate or capacity was defined as the mean percentage of the total translocated assimilate imported by the leaf in 24 hours. The relative export rate capacity was defined as the mean percentage of net photosynthate translocated from the source leaf in 24 hours. The experiment was performed 4 times.

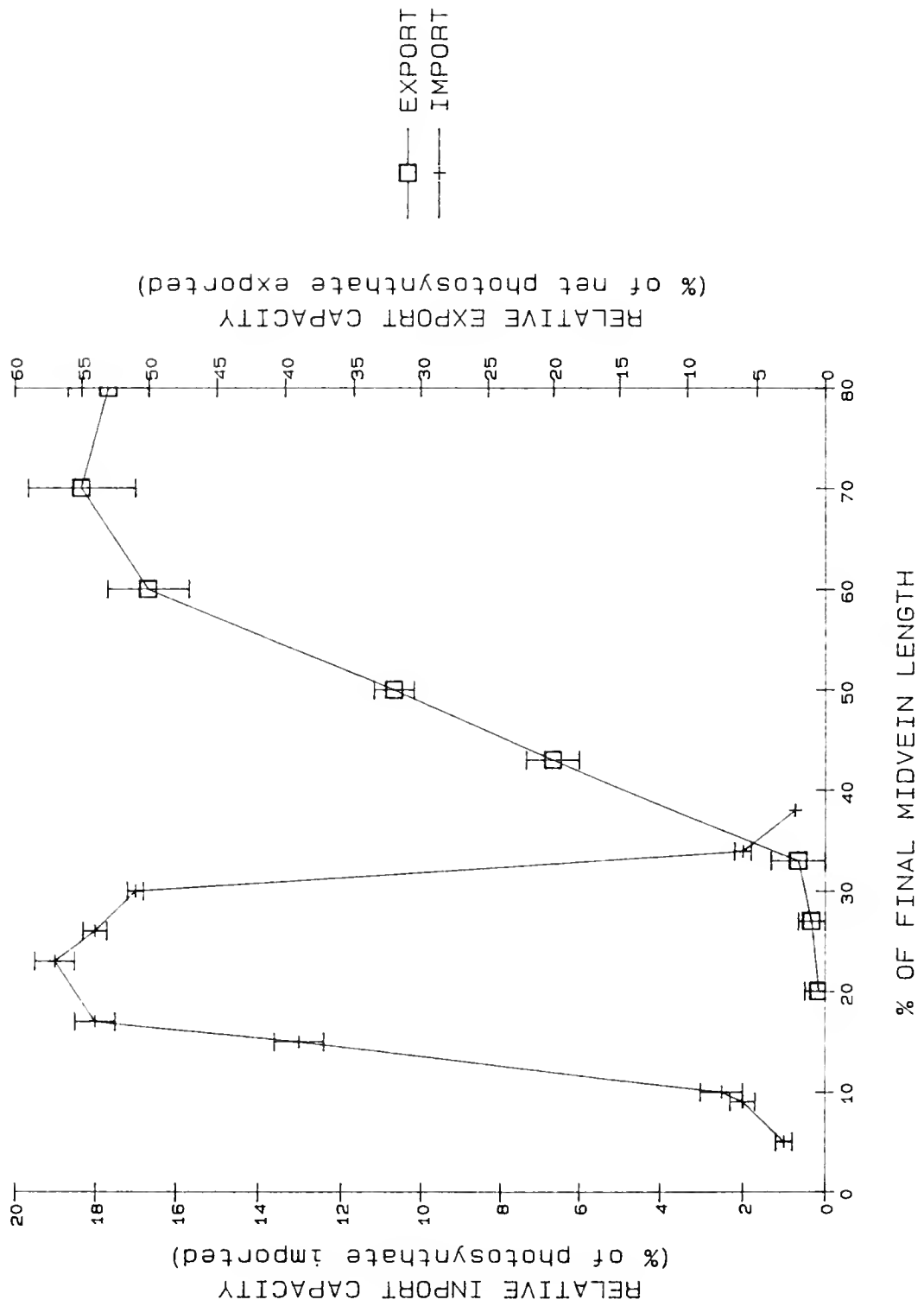


Figure 7-2. Early Season Labeling of Distal Leaves. All of the leaves distal to the inflorescence were labeled as described in the Materials and Methods. The branch was harvested immediately after labeling or 24 hours after labeling. The branch was dissected and processed for scintillation counting. The midvein length (mm) is the length of the midvein in the blade of the leaf. Leaf position describes the leaf's age and location. Leaf 1 is the most distal or acropetal; leaf 8 is the most proximal or most basipetal. All of these leaves are distal to the inflorescence. The experiment was repeated four times.

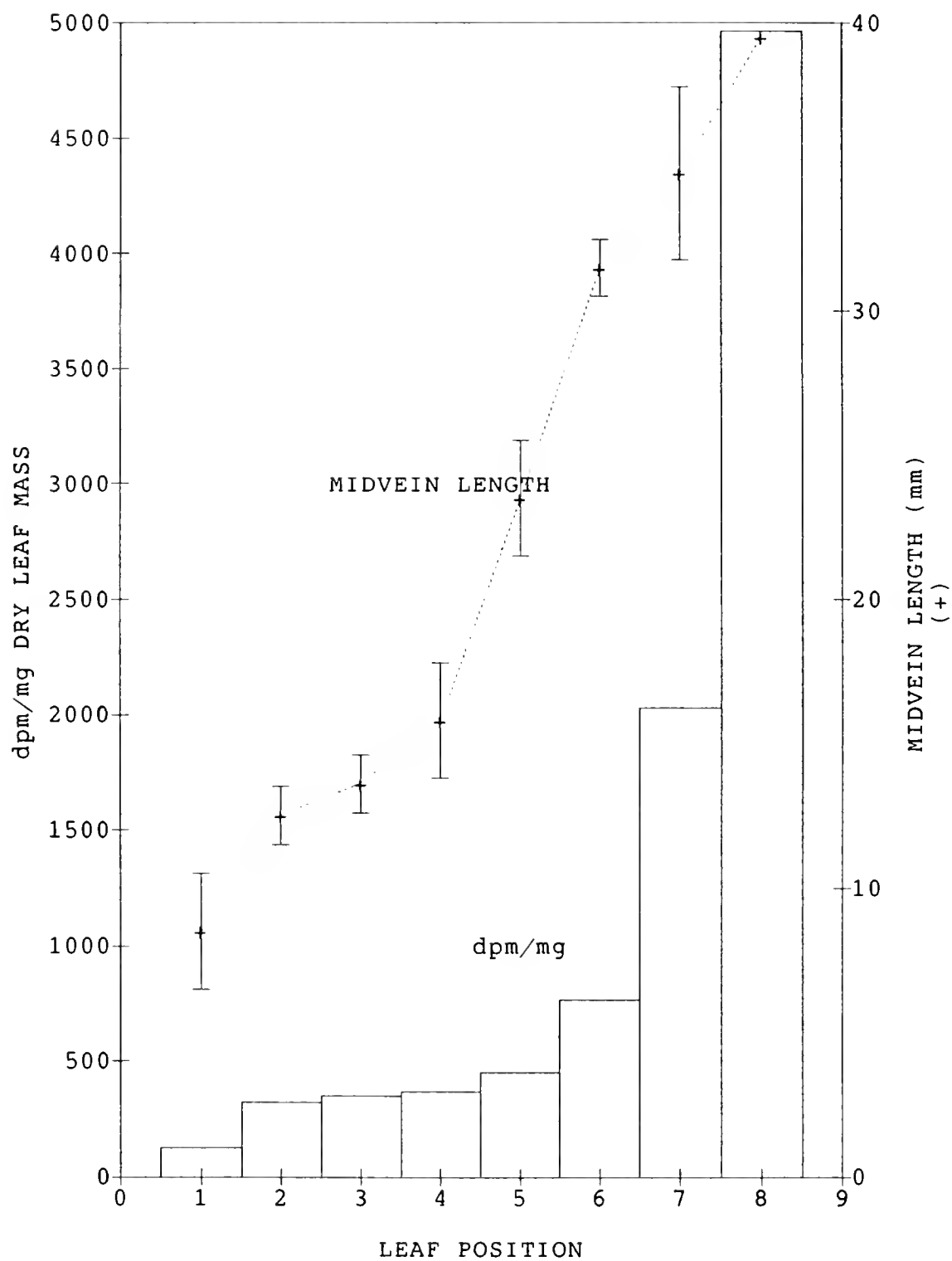


Figure 7-3. Distribution of Assimilates to Developing Distal Leaves when the Average Distal Midvein Length was less than 20 mm. The black-dotted leaf represents the radiolabeled leaf. The distribution of total radioactivity to each orthostichy is shown in this representative schmatic. These are the mean value from at least 10 replicates.

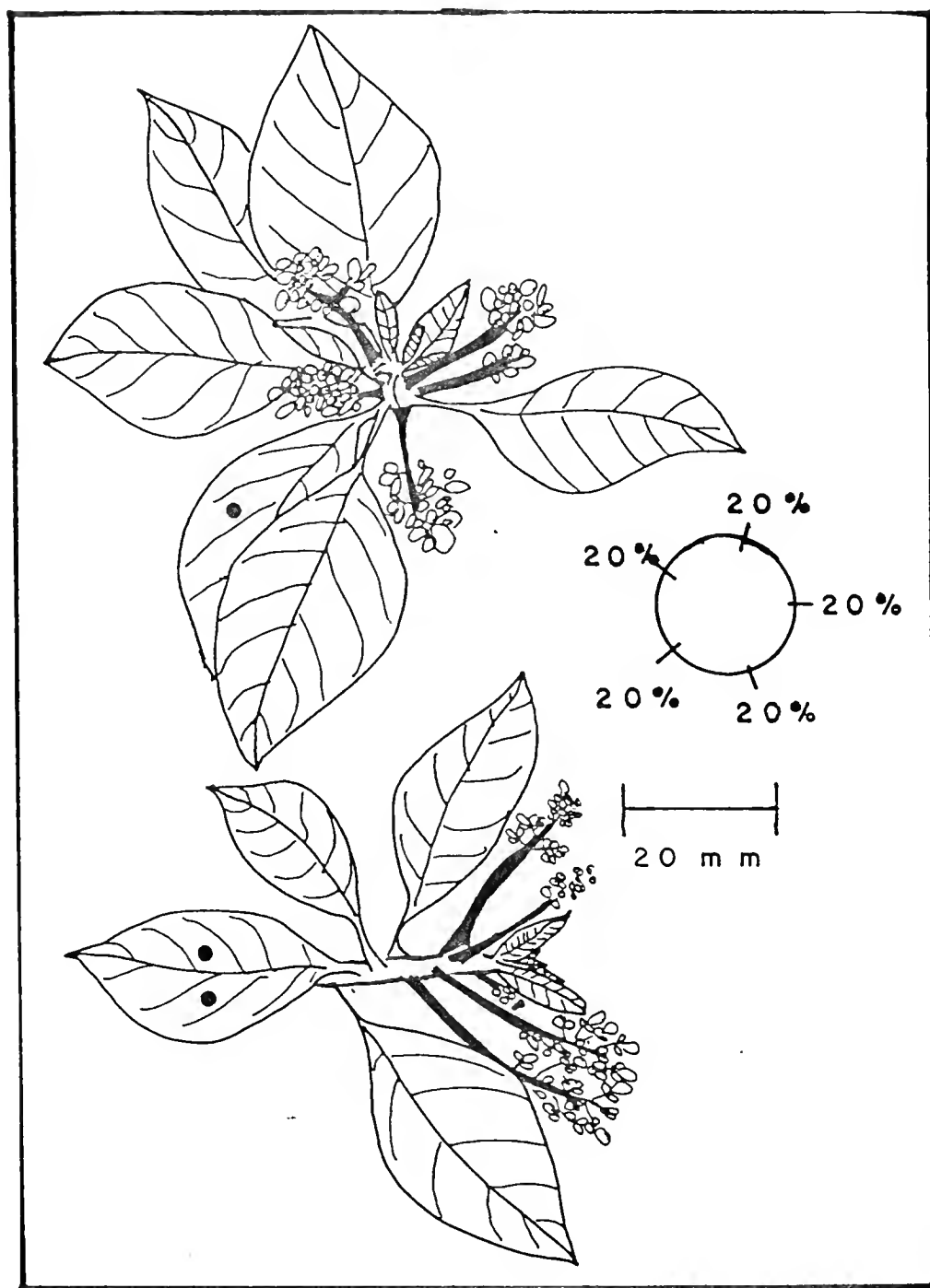


Figure 7-4. Distribution of Assimilates to Developing Distal Leaves when the Average Distal Midvein Length was between 20 and 30 mm. The distribution of total radioactivity to each orthostichy is shown in this representative schmatic. These are the mean values fron at least 10 replicates. The black-dotted leaf represents the radiolabeled leaf.

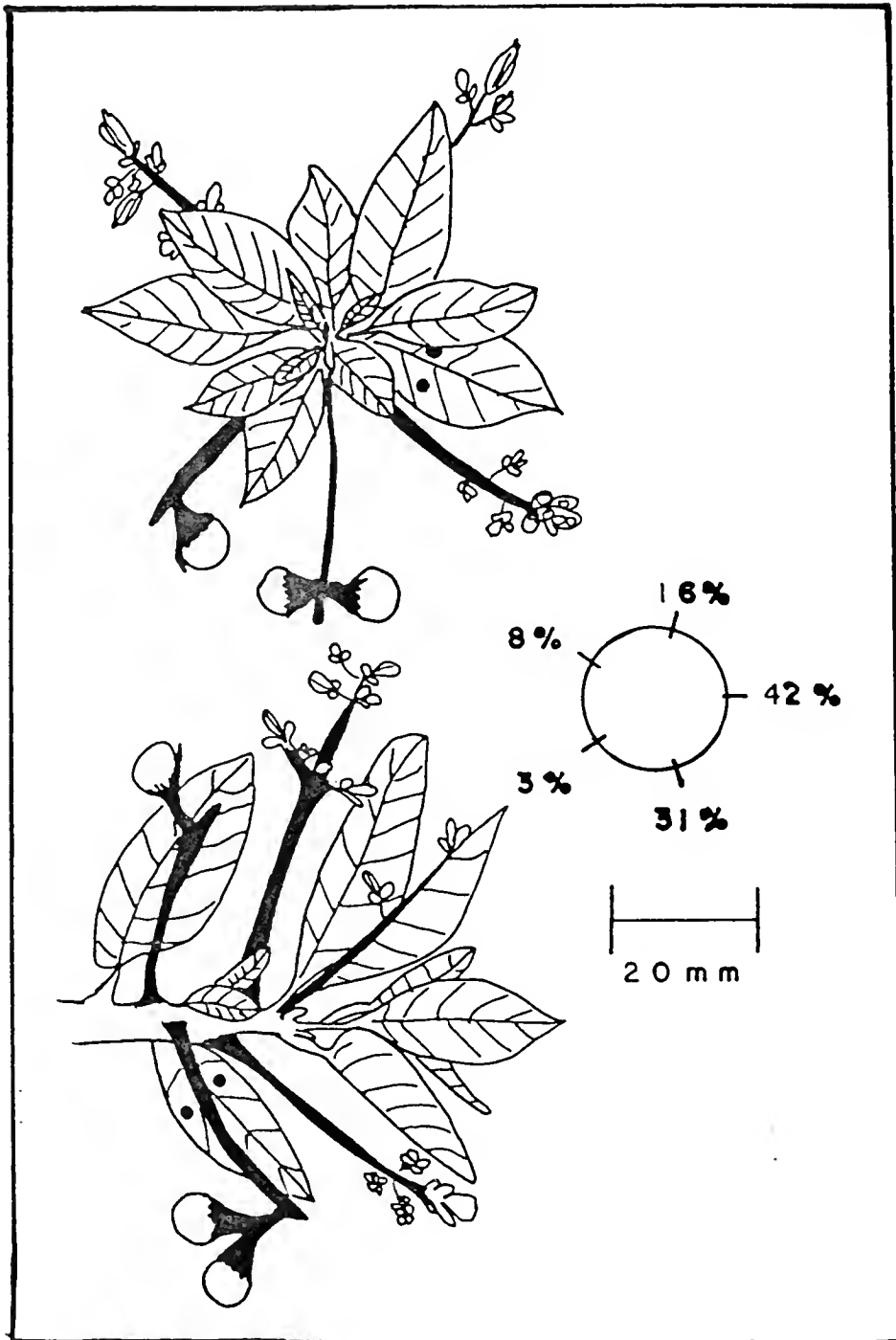


Figure 7-5. Distribution of Assimilates to Developing Distal Leaves when the Average Distal Midvein Length was between 30 and 40 mm. The black-dotted leaf represents the radiolabeled leaf. The distribution of total radioactivity to each orthostichy is shown in this representative schmatic. These are the mean values from at least 10 replicates.

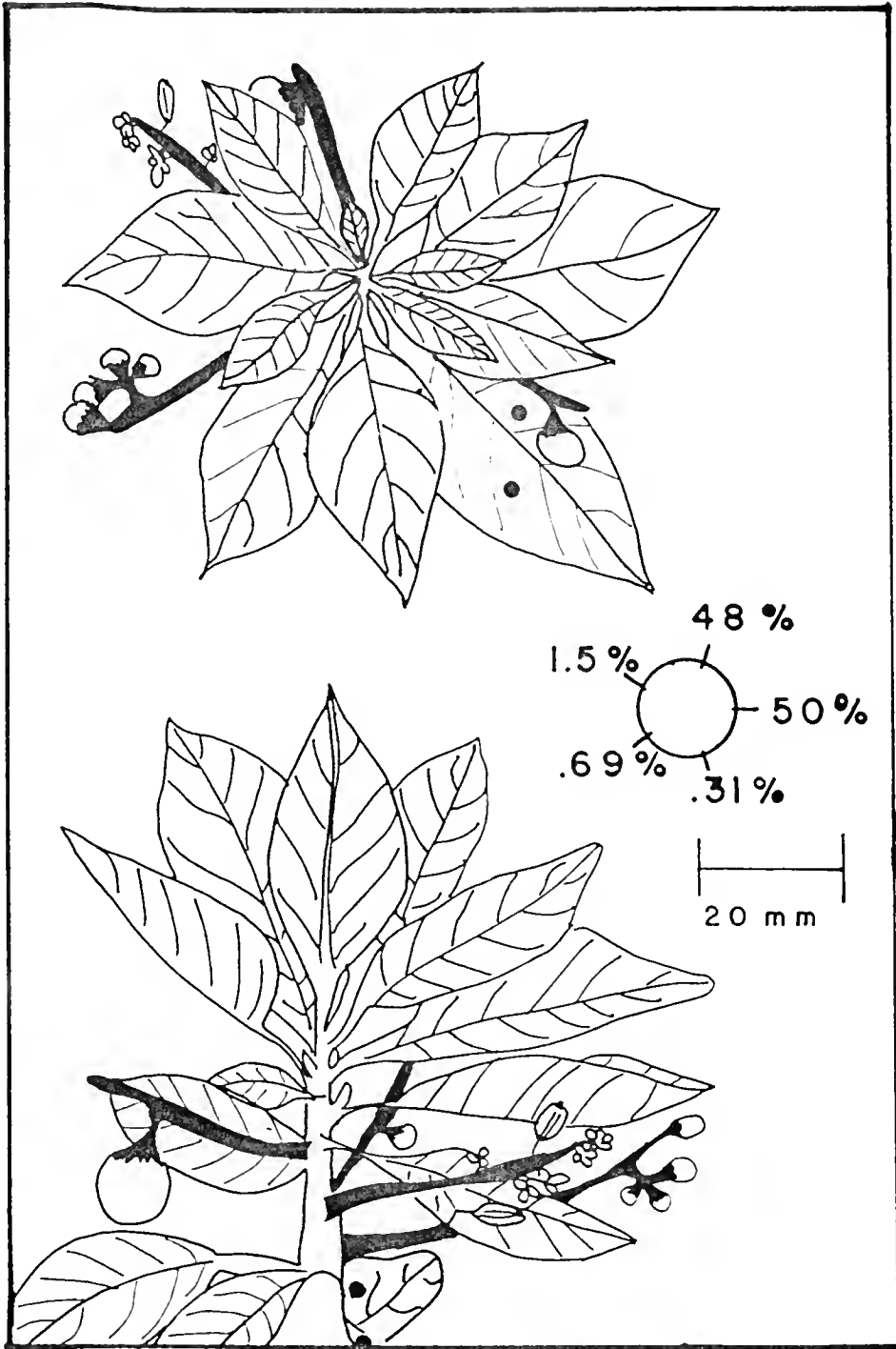


Figure 7-6. The Distribution of Assimilates to Fruits when there were no Fruit with the Same Phyllotaxy as the Source Leaf. A single leaf (black-dotted) distal to the fruits was labeled. The fruit were harvested and processed for scintillation counting 24 hours after labeling. The percentages indicate the mean proportion of the total fruit radioactivity associated with the fruit at each stelar location. The standard error for these measurements was 10% or less. This is a representative schematic which assumes fruit are present at 4 of the 5 stelar locations. Fifteen branches were used in this study.

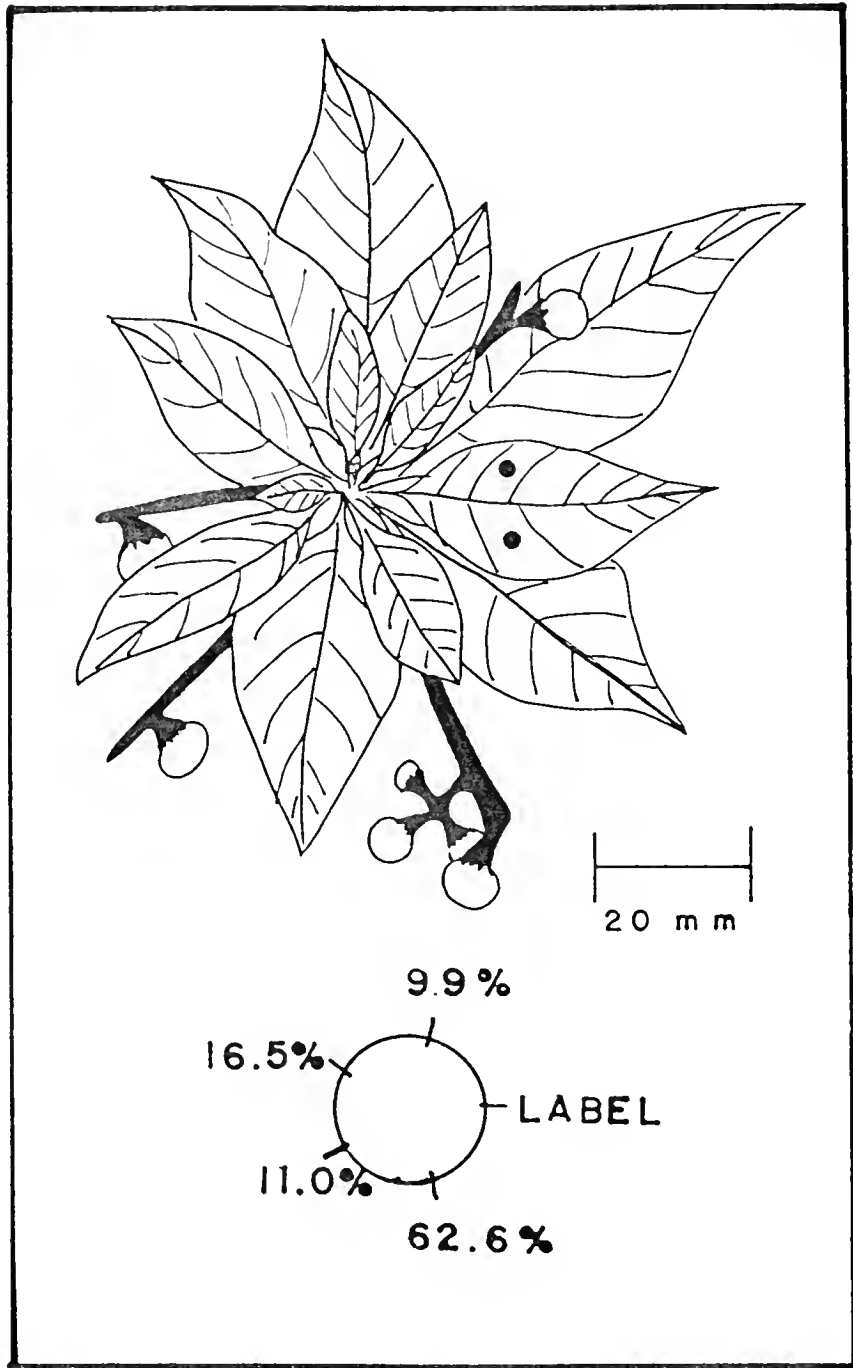


Figure 7-7. The Distribution of Assimilates to Fruits when the Source Leaf and a Fruit have the Same Phyllotaxy. A single leaf (black-dotted) distal to the fruits was labeled. The fruit were harvested and processed for scintillation counting 24 hours after labeling. The percentages indicate the proportion of the total fruit radioactivity associated with the fruit at each stelar location. The standard error for these measurements was 10% or less. This is a representative schematic which assumes fruit are present at each stelar location. Fifteen branches were used in this study.

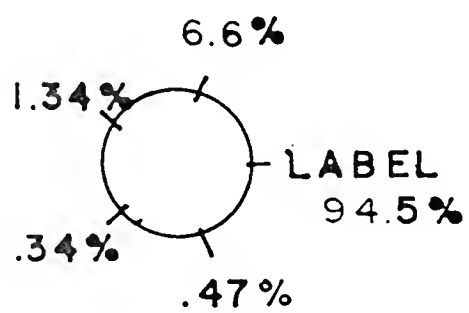
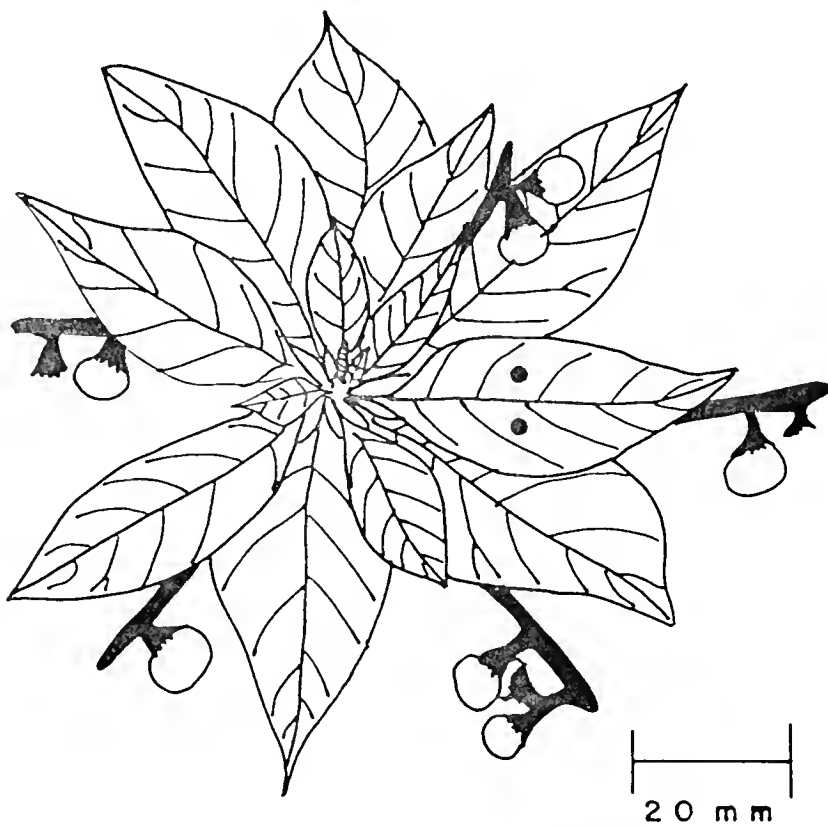
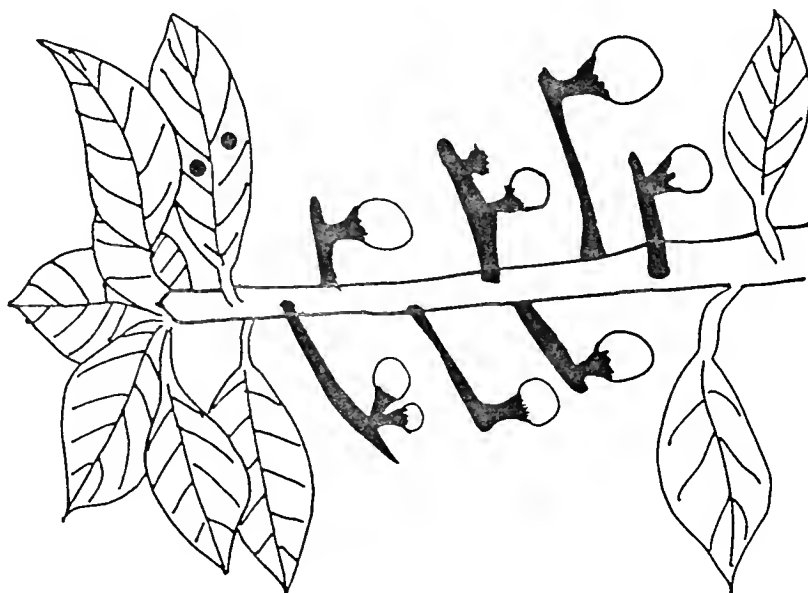


Figure 7-8. The Interaction of Mass and Linear Distance on the Distribution of Radiolabeled Assimilates. A single leaf distal to the fruit was labeled and fruit were processed as described in the Materials and Methods. Fruit number represents the fruit's linear proximity to the source leaf. Fruit 1 is closest to and fruit 8 is farthest from the source leaf. % C represents the amount of radiolabel recovered from the individual fruit as a proportion of the total radiolabel recovered from all of the fruit.



FRUIT NO.	DRY MASS	14 % C
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1	.0170	.021
2	.0275	2.100
3	.0492	17.700
4	.0401	.399
5	.0333	12.400
6	.0733	.310
7	.1289	27.800
8	.0438	39.300

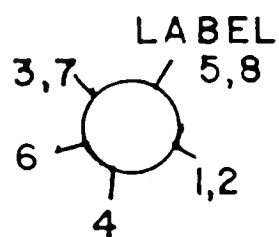
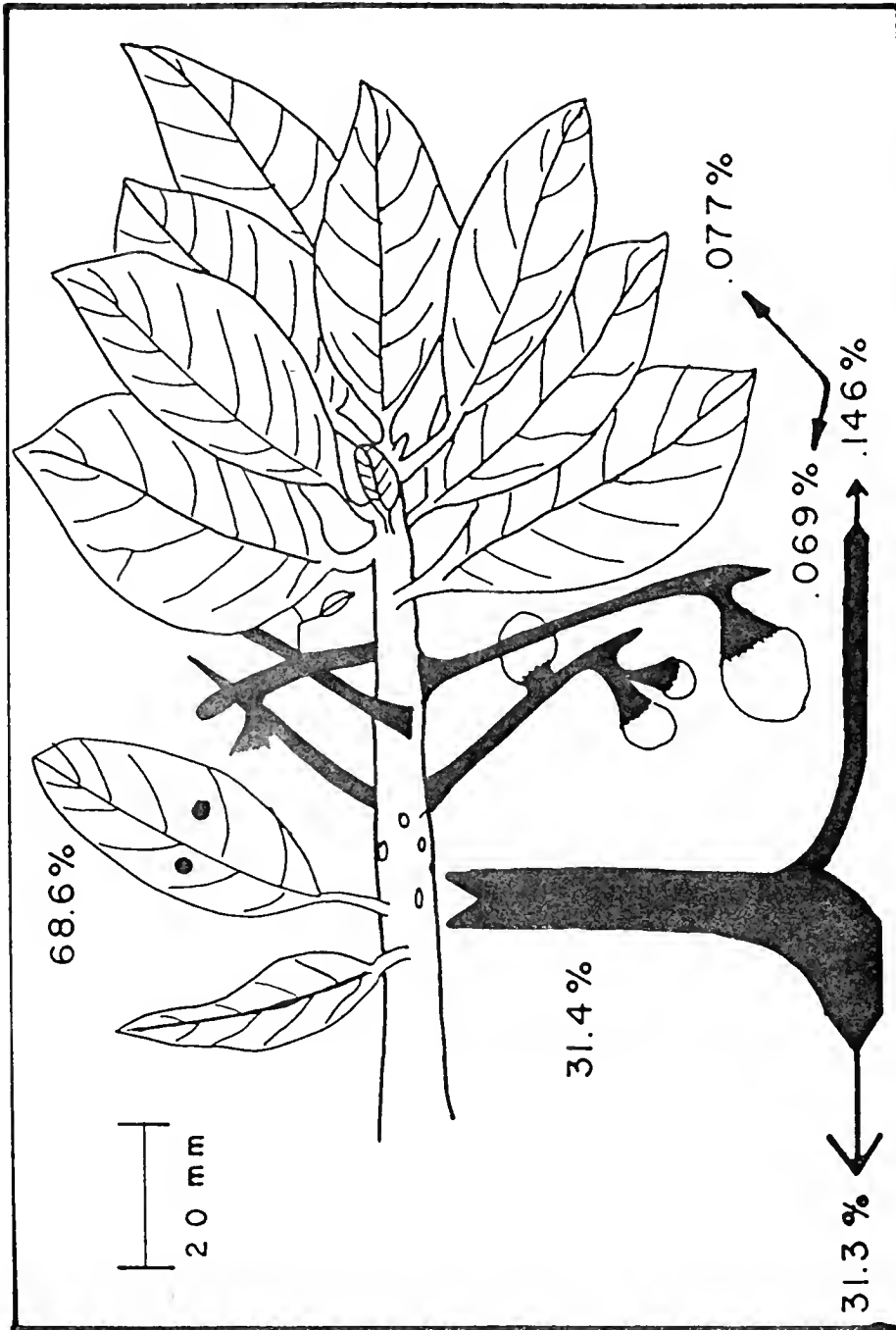


Figure 7-9. Translocation of Assimilates from Mature Leaves Distal to the Avocado Inflorescence. Within 48 hours a mean of 69% of the radiolabeled assimilates had been exported from the source leaf. Less than half of the exported assimilates were recovered in the fruit tissues. This is a representative schematic of at least 10 replicate experiments having a standard error of 5% or less.



CHAPTER 8
ASSIMILATION OF $^{14}\text{CO}_2$ BY NON-FRUIT BEARING FLUSHES OF AVOCADO:
PERSEA AMERICANA MILL. CV. 'PETERSEN'

Introduction

In avocado, leaf flush development and fruit development coincide for much of the season. Persea americana cv. Mill. may be either deciduous, semi-deciduous or evergreen. 'Petersen', the cultivar used in this study, is an evergreen variety. Two vegetative flushes occur between January and August in Florida. Individual leaves survive between 2 and 3 years. Reproductive flushes are first evident in late January. The inflorescence emerges in a pseudoterminal position. Leaves develop at the apex as the inflorescence expands. These developing tissues are supplied assimilates by the older leaves proximal to the inflorescence (Chap. 6). The leaves distal to the inflorescence mature and become the primary supplier of photoassimilates for the developing fruit and for another apical flush (Chap. 7). This final flush begins to develop in early June. Similar periods of flush development occur on non-fruit-bearing branches.

Research on starch reserves has focused primarily on stores in the roots and in the trunk (162). Although the leaves do not represent as large a reservoir for starch as these other tissues, they may play a significant role in plant development, particularly in evergreen trees. How the starch reserves in flushes of varying ages contribute to plant

growth is not known. Non-fruit-bearing branches of avocado represent a significant portion of the tree's foliage and their contribution to the tree's carbon economy may be significant.

This paper examines non-fruit-bearing branches, while a companion paper (Chap. 9) examines fruit-bearing branches of avocado. Photoassimilation by both branch types was examined to provide a direct comparison between fruit-bearing and non-fruit-bearing branches; since, the ultimate goal of this research was to determine if carbohydrate partitioning directly controls fruit abscission. The immediate goals of this research were three-fold. First, the assimilation and translocation of recently fixed photoassimilates by leaf flushes of different ages were measured to determine which flush was most efficient in fixing and translocating recently fixed photoassimilates. Second, the starch and sucrose content of the leaves was measured, so that pool sizes and pool turnover within the leaves of different flushes could be compared. Third, similar measurements were made in branches girdled 24 hours before labeling to determine what effect sink removal would have on carbohydrate metabolism.

Materials and Methods

Ten-year-old, field-grown trees of Persea americana Mill., cv. 'Peterson', located at the Tropical Research and Education Center, Homestead, FL were used in this study. The most distal 3 leaf flushes on a branch were enclosed in a sealed plastic bag and labeled with 100 μCi of $^{14}\text{CO}_2$ for a one-hour period between 0900 and 1100 h $^{14}\text{CO}_2$ was generated from $\text{NaH}^{14}\text{CO}_3$ (Amersham with a specific activity of 54

mCi/mmol) using equimolar amounts of 5% lactic acid. Three branches were chosen and individually labeled as described above for each experiment. Branches were chosen from the upper, middle and lower canopy positions at each labeling. The experiment was performed at least 3 times. The most distal flush will be designated as flush 3, the flush proximal to flush 3 will be designated as flush 2 and the most proximal flush will be flush 1.

Translocation from the leaves was measured by taking .309 cm² leaf punches throughout the experimental period. Each leaf was sampled twice daily during the experiment. The entire branch was harvested 70 hours after labeling. The leaf samples were dried, weighed and digested with a commercial tissue solubiliser (Scintigest - Fisher Scientific). The tissues were decolorised with H₂O₂ as described by Burrell and Brunt (25). The sample vials were then filled with scintillation fluid and counted.

The metabolite pool was studied by examining the proportion of radioactivity in the acid, neutral and basic fractions of the ethanol extract. Leaf punches were taken at various times and packed in ice for transport to the laboratory. Flush samples were pooled and boiled in 85% ethanol. Samples were processed after the method of Housely et al. (102). The residue from this extraction was used to determine the starch content by the phenol-sulfuric acid method (53).

Results and Discussion

The importance of carbohydrate levels on flowering and fruiting has been reviewed by others (45). Generally research on the carbohydrate levels in perennial tree crops has focused on seasonal

fluctuations in the starch levels of the roots, trunk and branches of the tree (5,11,16,23,42). Work by Scholefield et al. (162) and Cameron and Borst (26) has shown that in avocado carbohydrate levels, specifically the starch levels in the branches, trunk and roots, decrease sharply during flowering and fruit development. A decrease in total carbohydrates has been associated with alternate bearing in "off season" avocado trees. Fruit removal (161) increased leaf dry mass and increased the number of starch grains in the most recent flush of leaves.

The presence of multiple leaf flushes concurrently on a branch during fruit development provides an interesting model for carbohydrate partitioning with respect to leaf age, leaf position and fruit load. Even though fruit load is not specifically addressed in this paper the contribution of the non-fruit-bearing branches to the tree's carbon economy may be significant and warrants investigation. Eight branches, each bearing three mature flushes of leaves were chosen for these experiments. Flush 3, the most recently matured flush, consisted of 11 ± 2.9 leaves, averaging 86 ± 4 mm in length (lamina), and 304 ± 45 cm² in area. The values for Flush 2, the early spring flush and Flush 1, the flush formed during the previous season were very similar to those of Flush 3. The total leaf area of flushes on non-fruit-bearing branches was generally greater than on fruit-bearing branches (Chap. 9). Defruited and non-fruit-bearing plants have greater total leaf areas than the corresponding fruit-bearing plants (5,160).

The most recent flush (Flush 3) assimilated the greatest proportion of the available radiolabel (Fig. 8-1). Flush 3 incorporated 50% of the total label fixed by the branch. Flush 2

incorporated 30% and flush 1, 20% of the total label incorporated by the branch. The youngest, most recently formed flush therefore accounted for the majority of the carbon fixed by the branch. The age dependent decrease in photoassimilation has been linked to a decrease in the soluble and fraction 1 proteins and to a decrease in RuBPcase activity (82,137). These observations have been made primarily in annual agronomic crops (82,137) where age is determined by canopy position. However, in evergreen perennials such as avocado, flush development probably provides the best correlation for leaf age and physiological activities.

When the branches were girdled the differences in carbon fixation between the flushes were accentuated with Flush 3 assimilating approximately 65% of the label while Flush 1 assimilated less than 15% of the label. Although girdling caused an apparent increase in the relative fixation of $^{14}\text{CO}_2$ by leaves in Flush 3, it decreased absolute fixation by all three flushes (Fig. 8-2). Flushes on non-girdled branches had greater specific activities, whether compared on a dry weight (Fig. 8-2) basis or an area (Fig. 8-3) basis. On girdled branches the specific activities of the leaf flushes decreased particularly for the older flushes. Girdling within 24 hours of labeling adversely affected the assimilation of $^{14}\text{CO}_2$ and skewed the relative efficiency of fixation in favor of the youngest flush. Decreasing the sink to source ratio has been shown to increase leaf and intercellular resistances and decrease carbon fixation (83,118).

An inverse relationship, with the oldest flush having lowest values and the youngest flush having the highest values, existed between assimilation and leaf mass or leaf area. The distal flush was

the least massive (Fig. 8-4) yet it had the greatest values for the dpm fixed per area and dry mass. As the plastochron leaf age increased, leaf mass increased and the relative dpm content decreased. The relative decrease in assimilation observed between flushes of different ages probably resulted from the decrease in soluble and fraction 1 proteins (82,83) combined with mass increases associated with an increase in starch and structural components. Starch and other structural carbohydrates add to the leaf mass but did not enhance photoassimilation.

In non-girdled flushes between 42-61% (Fig. 8-5) of the initially fixed ^{14}C remained in the leaves after 21 hours. The oldest flush had the slowest rate of translocation, translocating 25% of the fixed label in 21 hours, while the youngest flush had the greatest rate of translocation, translocating 50% of the fixed label during the same time period. The rate of translocation has been correlated to net photosynthesis (184). Rapid photosynthesis usually accompanies rapid translocation and rapid utilization. Girdling branches decreased translocation from the flushes by almost 50%. At 70 hours the non-girdled branches had exported approximately 72% of the initially fixed CO_2 while girdled branches had exported only about 29% of the initially assimilated label. Fondy and Geiger (67) described lower rates of translocation in girdled bean leaves than in control plants. They suggested that since the net carbon exchange rate did not change, that girdling alters the distribution of photoassimilates to various sink/pools.

The relative percentages of label in the neutral (sugars), basic (amino acids), acid 1 (organic acids and sugar monophosphates), acid 2 (phosphoglyceric acid and sugar diphosphates) fractions were not

significantly different between flushes at specific time points (Table 8-2). This suggested that recent photoassimilates were incorporated into various soluble pools in the same proportions.

Similar distributions of sucrose were noted in both girdled and non-girdled branches when based on area measurements (Fig. 8-6). The sucrose specific activity (Fig. 8-7) values for the distal flushes were identical on both the girdled and non-girdled branches suggesting that girdling within 24 hours did not negatively affect labeling kinetics or pool sizes in the youngest leaves. The second flush contained the most sucrose by area and the first flush the least sucrose. Girdling appeared to have little effect on sucrose specific activity (Fig. 8-7), except for slightly elevated values found in the leaves of the older flushes (1 and 2) of the girdled branches. The decreased translocation rate and the smaller sucrose pool size in these older flushes (Figs. 8-6, 8-8), coupled with the incorporation of label, even at the reduced rate noted in girdled branches, would elevate the pool specific activity. Alternatively, the older flushes on the girdled branches could act as an alternate sink or storage area (29, 39) for assimilates translocated from the younger flushes since girdling the branch removed the branches primary sink. This second alternative is supported by increased sucrose content observed in the older flushes of girdled branches.

In longer term, batch studies the leaves on girdled, non-fruit-bearing branches accumulated 50% more starch than similar non-girdled non-fruit-bearing branches (data not shown). Starch content of the leaves on the girdled branch peaked 6 days after girdling. The specific activity of starch extracted from the leaves of

girdled branches declined with time. The decline in the specific activity of starch extracted from the leaves paralleled the increase in starch content. The increase in starch during the course of the experiment diluted the radiolabeled pool. These leaves contained between 27 and 52 mg starch/gm tissue at the peak and low point of their diurnal cycle.

When this preliminary work was expanded to examine individual flushes, it was found that the relative starch content was greatest in the youngest flush (Fig. 8-9). When branches were girdled the relative distribution of starch shifted in favor of flush 3 and 2. This was not surprising since these flushes initially assimilated 90% of the available label. The specific activities of starch (Fig. 8-10) extracted from the leaves was greater for the flushes on the non-girdled branches than for their girdled counterparts. Likewise, the specific activity values for starch extracted from flush 3 were greater than for flush 1 or 2.

Avocado provides an interesting model for assimilate partitioning for many reasons, not the least of which is the presence of multiple leaf flushes on a branch during fruit development. Assimilation and translocation in non-fruit-bearing branches exhibited an age dependent correlation. The youngest, mature flush fixed the greatest proportion of available label and translocated the recently fixed assimilate the fastest. Although the relative pool sizes (Table 8-1) were the same for each flush the absolute sucrose content was greatest in the youngest flush. The relative starch content of the youngest flush was also greater than the other two flushes. Girdling decreased the absolute assimilation of label by all of the flushes and decreased the

relative distribution of sucrose and starch within the leaves of the branch. Girdling the branch increased the sucrose content, sucrose specific activity and starch content of the oldest flush. Although mature leaves lose the ability to actively import sucrose (73), the present data suggested otherwise. In conclusion, the age of leaves on non-fruit-bearing branches did affect photoassimilation and the rate of translocation with the youngest leaves assimilating and translocating the majority of the recent radiolabelled photoassimilate.

Table 8-1. Relative Pool sizes of Soluble Radiolabeled Compounds in Avocado Leaves.

Samples were taken from leaves at various times points and processed by the method of Housely et al. (102). The neutral fraction represents the sugars; the basic fraction consists of amino acids; organic acids and sugar monophosphates are included in the acid 1 fraction; phosphoglyceric acid and sugar diphosphates are included in the acid 2 fraction. These results are presented as percentages of the total radioactivity recovered from the columns. Time is given in hours post-labeling.

Time	Neutral	Basic	Acid 1	Acid 2
0	88.8 +/- 4.1	8.56 +/- 4.2	1.53 +/- 1.6	0.12 +/- 0.02
24	86.6 +/- 6.6	6.5 +/- 1.7	4.37 +/- 2.3	3.47 +/- 2.5
52	83.3 +/- 6.3	9.5 +/- 0.2	4.82 +/- 4.0	2.18 +/- 1.8
78	76.7 +/- 7.5	11.27 +/- 1.7	4.95 +/- 4.0	5.32 +/- 3.6

Figure 8-1. Relative Distribution of ^{14}C -Labeled Assimilates in Three Successive Avocado Leaf Flushes. Leaf samples (0.618 cm^2) were taken from each leaf of each flush on both girdled and non-girdled branches immediately after the 1 hr labeling period. These samples were dried, weighed, digested and the radiolabel quantified as described in the Material and Methods. At the end of the experiment, branches were harvested and leaf area was measured. Leaf area and label incorporation after 1 hr. exposure to $^{14}\text{CO}_2$ were used to calculate the proportion of label incorporated by each of three consecutive, girdled or non-girdled flushes. Branches were approximately 70-80 cm in total length and 1 to 1.5 cm in diameter. Branches were girdled proximal to the oldest flush, adjacent to the main scaffolding branch. Three branches were chosen, 1 each from the lower, middle and upper canopy positions. The experiment was repeated four times.

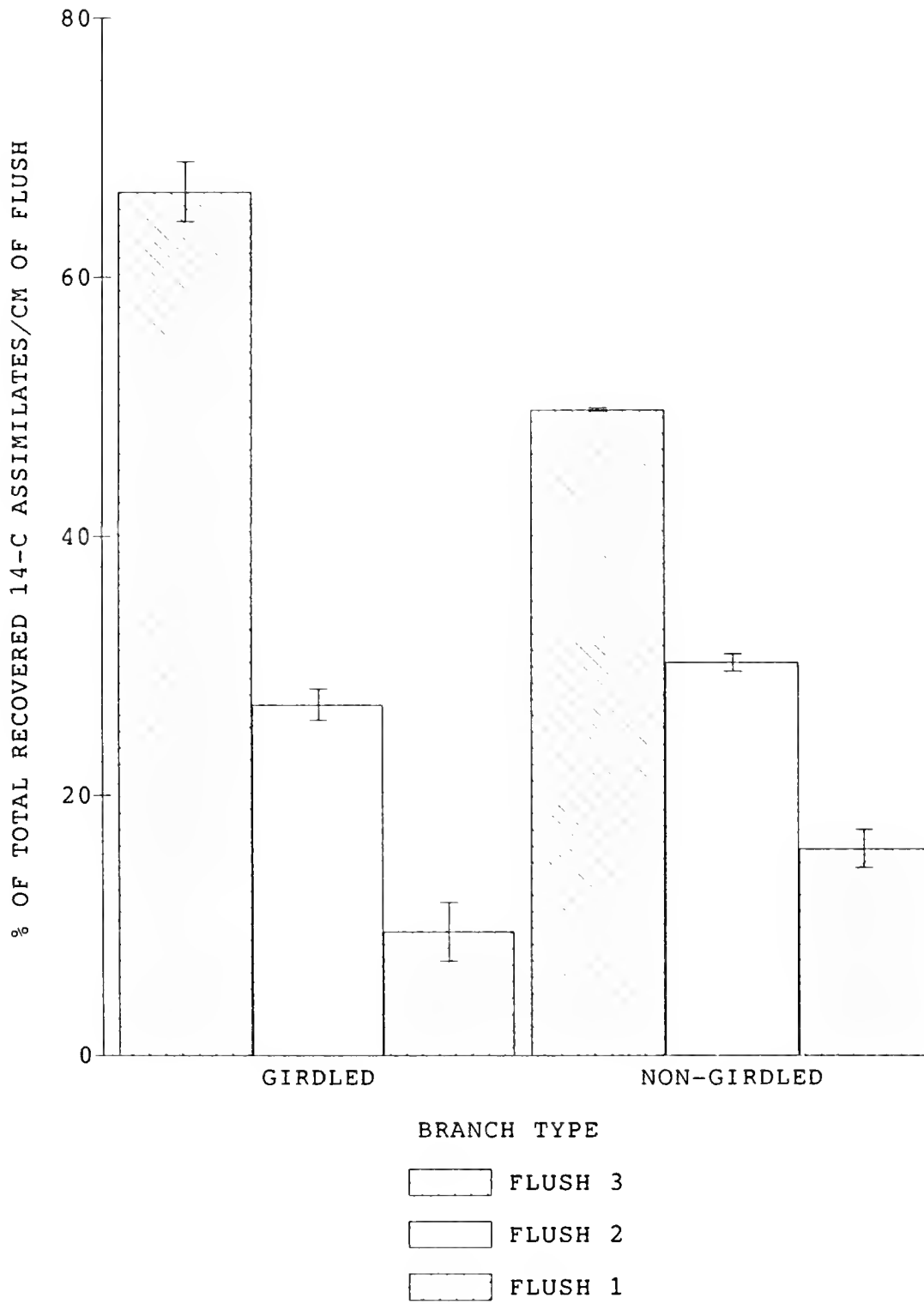


Figure 8-2. Specific Activity of Avocado Leaf Tissue from Three Successive Leaf Flushes. Leaf samples were taken from each leaf of each flush on both girdled and non-girdled branches immediately after the 1 hr labeling period. These samples were dried, weighed, digested and the radiolabel quantitated as described in the Materials and Methods. Leaf disk (0.618 cm^2) dry mass and label incorporation after 1 hr. exposure to $^{14}\text{CO}_2$ of all the leaves in the flush were used to calculate the mean incorporation of label by each of three consecutive, girdled or non-girdled flushes. Branches were approximately 70-80 cm in total length and 1 to 1.5 cm in diameter. Branches were girdled proximal to the oldest flush, adjacent to the main scaffolding branch. Three branches were chosen, 1 each from the lower, middle and upper canopy positions. The experiment was repeated four times.

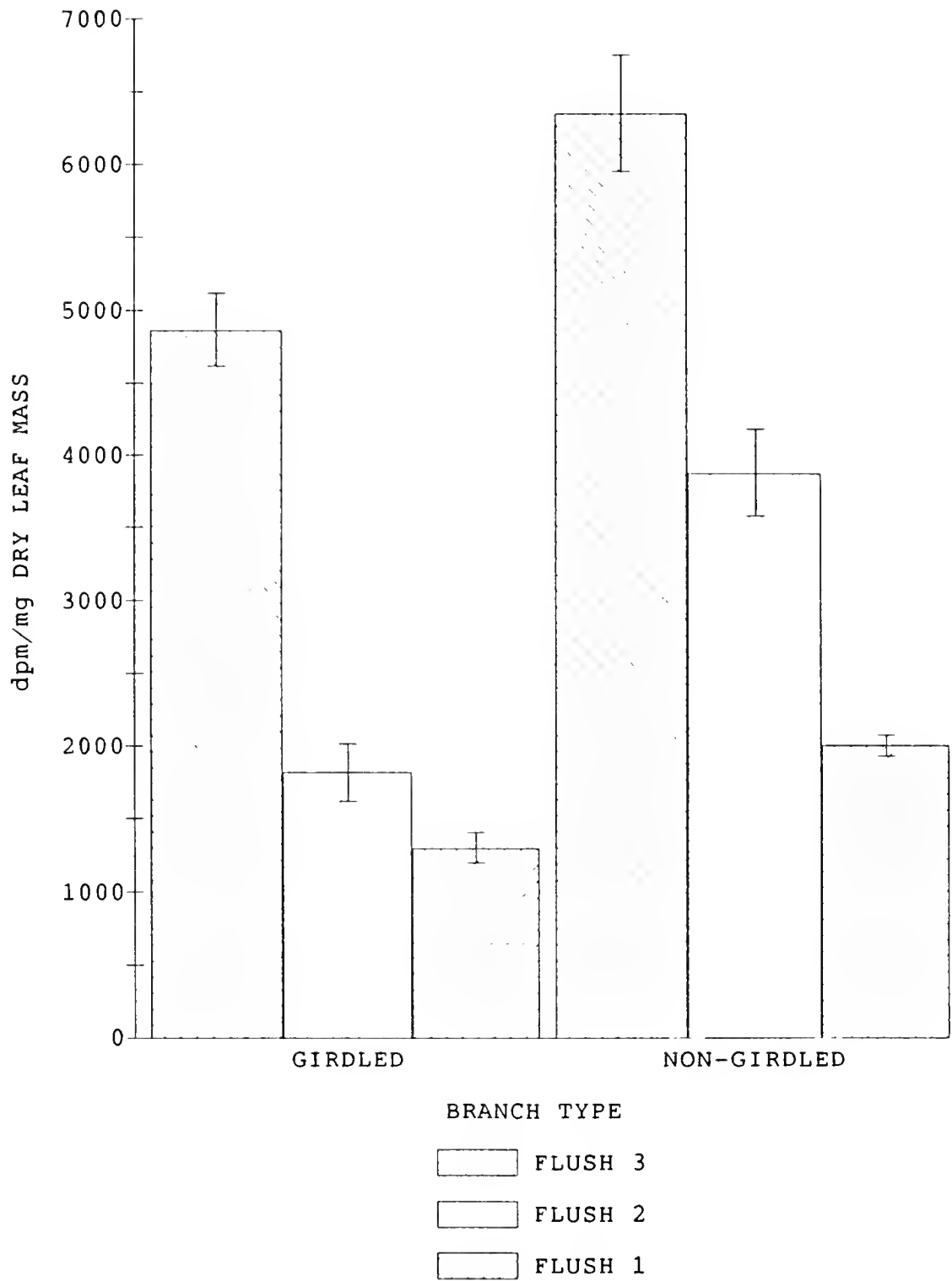


Figure 8-3. Radioactivity per Unit Avocado Leaf Area. Leaf samples (0.618 cm^2) were taken from each leaf of each flush on both girdled and non-girdled branches immediately after the 1 hr labeling period. These samples were dried, weighed, digested and the radiolabel quantified as described in the Material and Methods. At the end of the experiment, branches were harvested and leaf area was measured. Leaf area and label incorporation after 1 hr. exposure to $^{14}\text{CO}_2$ were used to calculate the dpm/cm^2 of label incorporated by each of three consecutive, girdled or non-girdled flushes. Branches were approximately 70-80 cm in total length and 1 to 1.5 cm in diameter. Branches were girdled proximal to the oldest flush, adjacent to the main scaffolding branch. Three branches were chosen, 1 each from the lower, middle and upper canopy positions. The experiment was repeated four times.

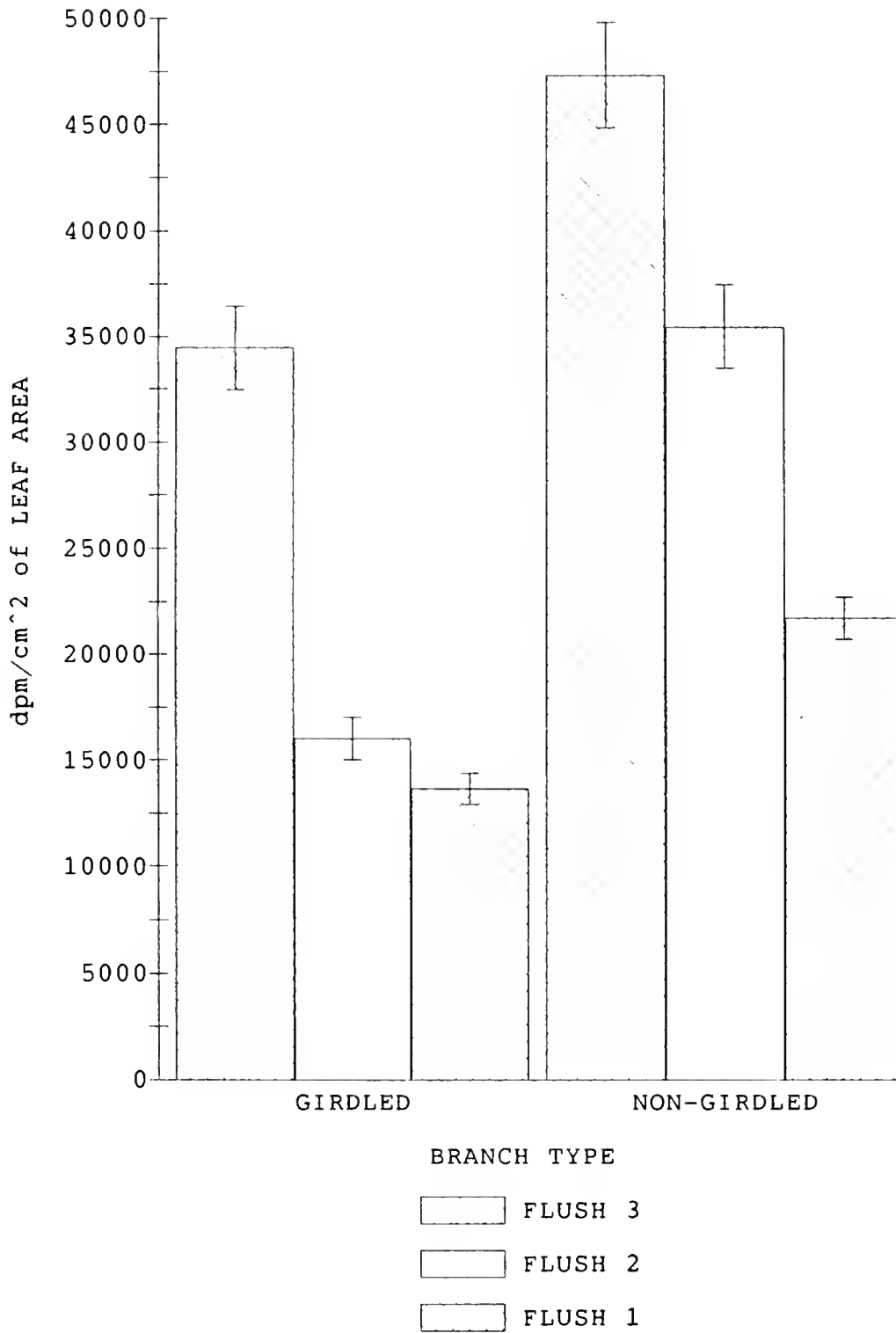


Figure 8-4. Specific Leaf Weight of Avocado Leaves from Three Successive Growth Flushes on Girdled and Non-Girdled Branches. Leaf disks (0.618 cm^2) were removed from each leaf of each flush on both girdled and non-girdled branches. The leaf disks were dried and weighed as described in the Materials and Methods. Each flush bore at least 10 leaves and each leaf was sampled at least 4 times during the experiment.

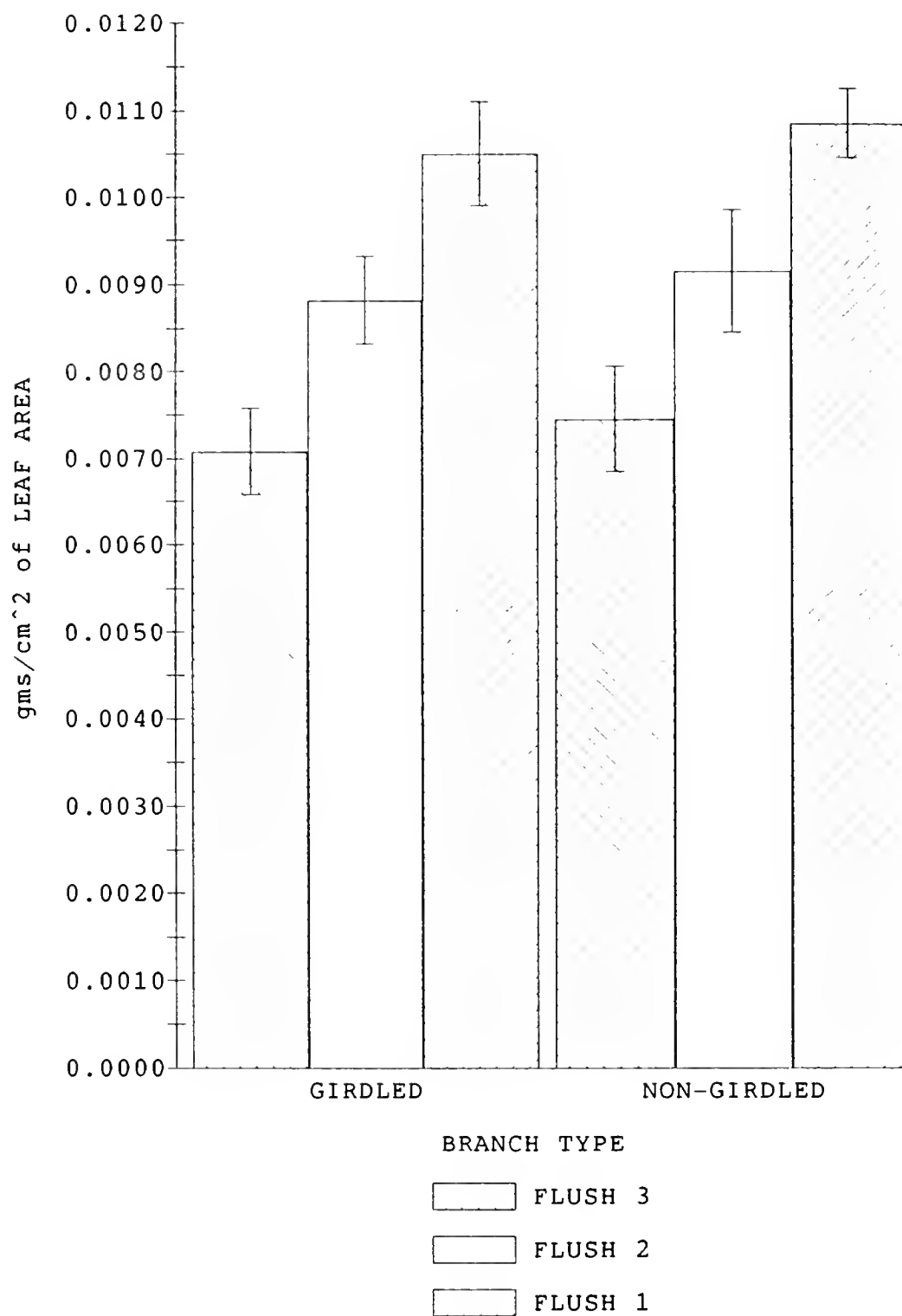
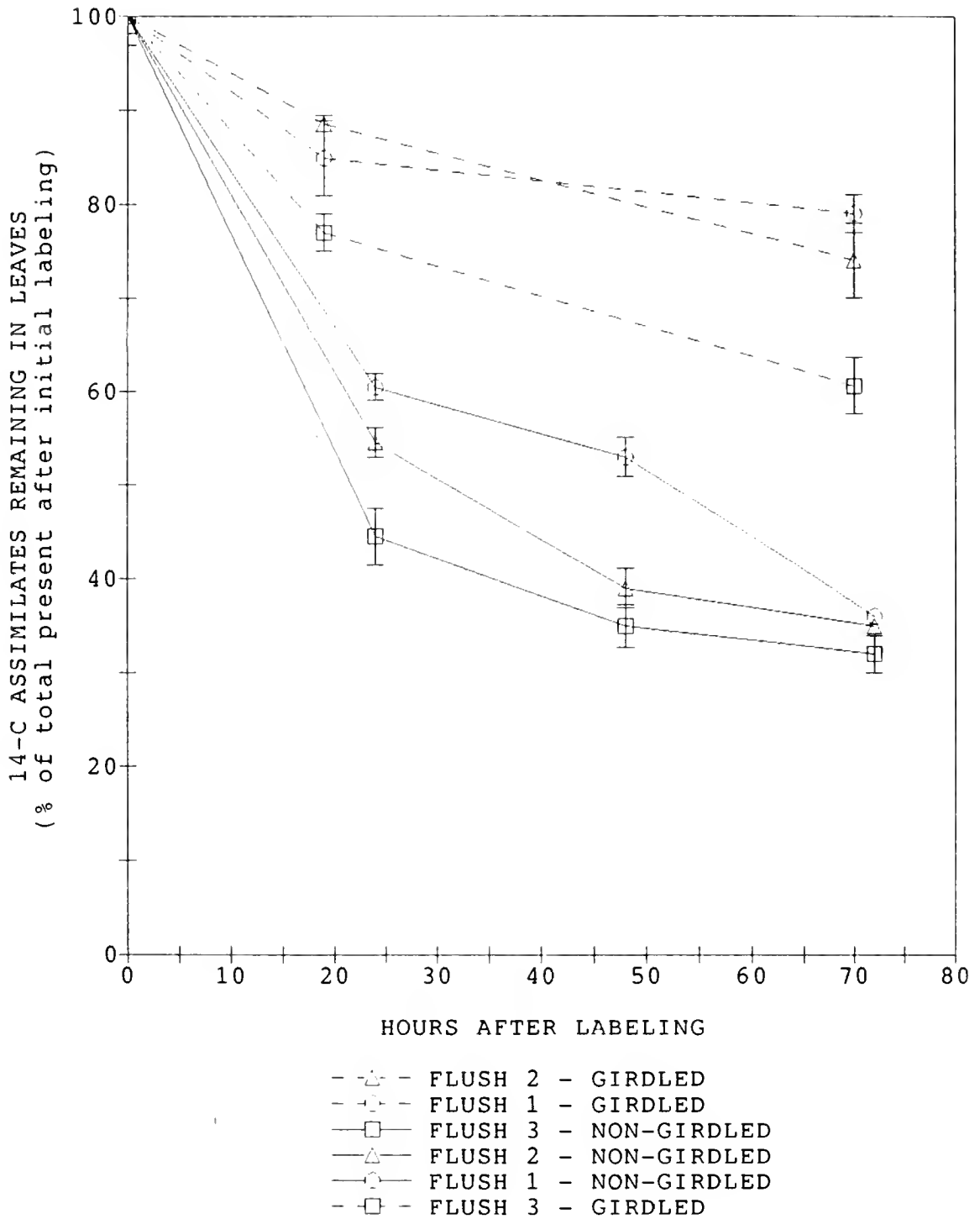


Figure 8-5. Export of Labeled Assimilates form Leaves of Three Successive Growth Flushes on Girdled and Non-Girdled Avocado Branches. Leaf disks taken throughout the experimental period were dried, weighed and digested as described in the Materials and Methods. Translocation was defined as the difference between the amount of radiolabel present immediately after a 1 hr pulse of $^{14}\text{CO}_2$ and the given time point during the 75 hr chase.



NEXT

Figure 8-6. Sucrose Content per Unit Area of Avocado Leaves from Three Successive Growth Flushes on Girdled and Non-Girdled Branches. Leaf disks (0.618 cm^2) were removed from each leaf of each flush at harvest. The samples from each flush were pooled, chopped and boiled in 85% EtOH. The ethanolic extracts were concentrated and the sugar content was measured by the phenol-sulfuric acid method.

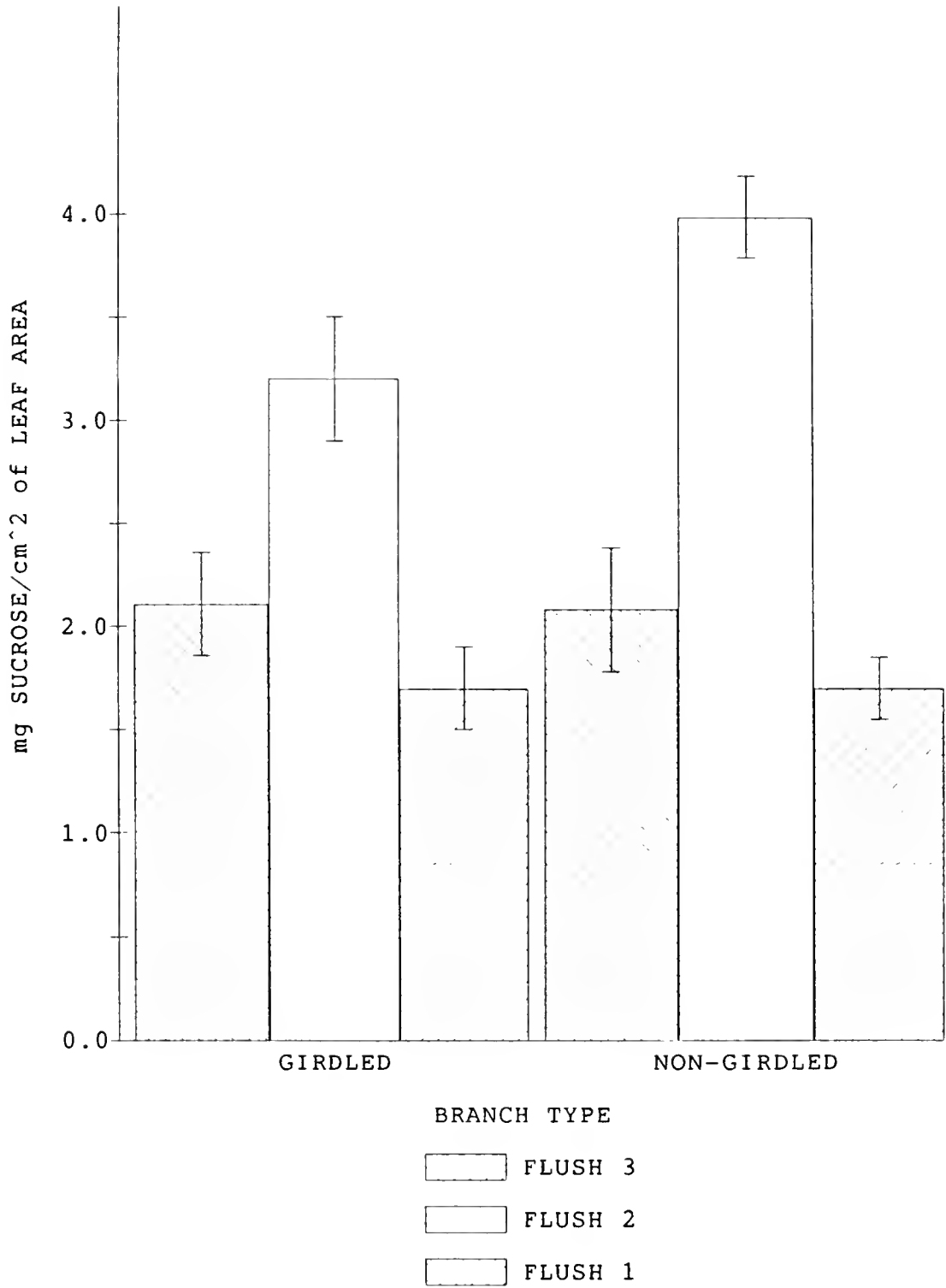


Figure 8-7. Specific Activity of Sucrose Extract from Avocado Leaves of Three Successive Flushes on Girdled and Non-Girdled Branches. At harvest, leaf disks (0.618 cm^2) were removed from each leaf of each flush. The samples from each flush were pooled, chopped and boiled in 85% EtOH. The ethanolic extracts were concentrated. Sugar content was quantitated by the phenol-sulfuric acid method. Samples of known sucrose concentration were decolorized for scintillation counting.

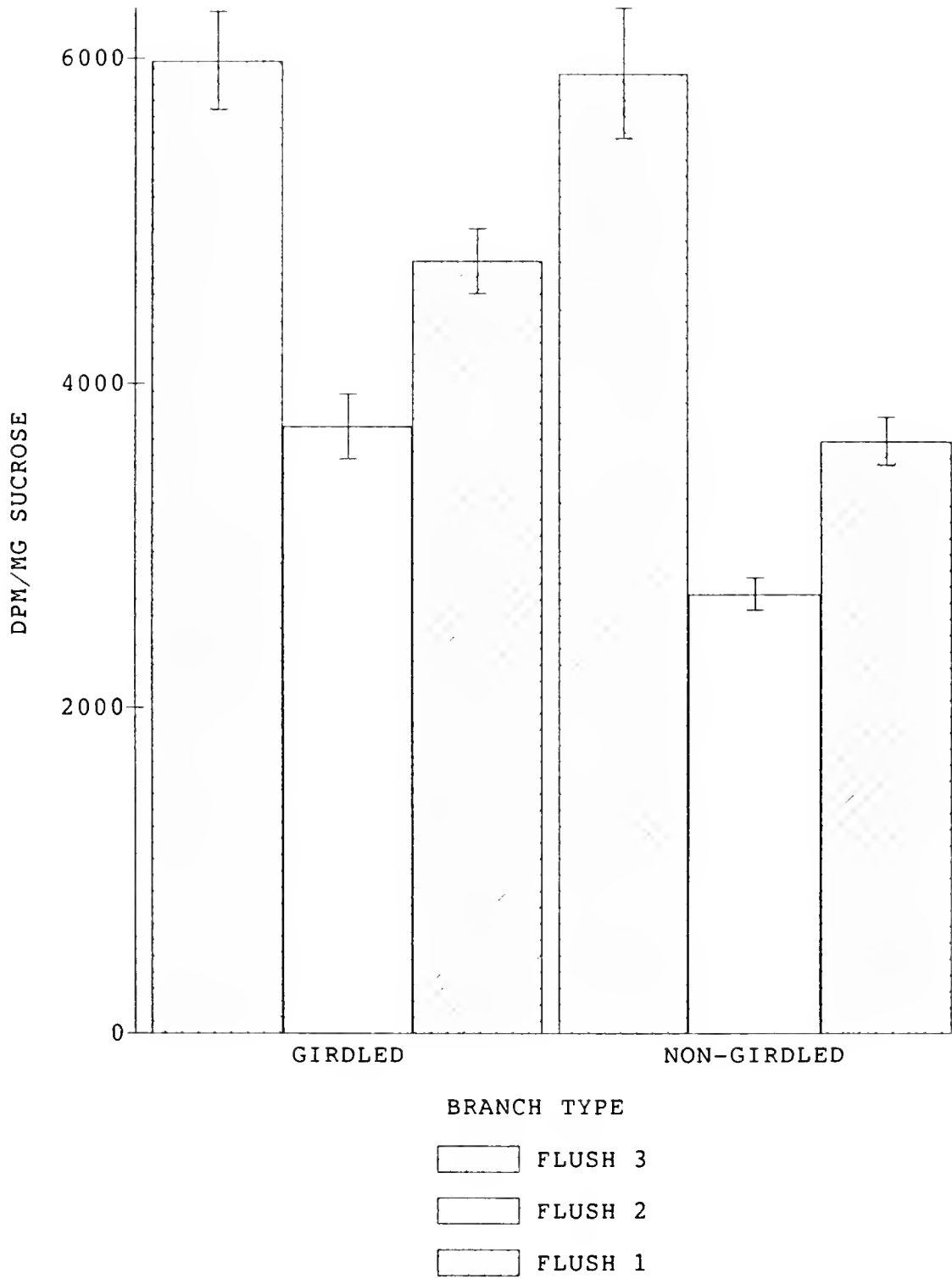


Figure 8-8. Total Sucrose Content of Avocado Leaves from Three Successive Growth Flushes on Girdled and Non-Girdled Branches. Leaf disks (0.618 cm^2) were removed from each leaf of each flush at harvest. The samples from each flush were pooled, chopped and boiled in 85% EtOH. The ethanolic extracts were concentrated and the sugar content measured by the phenol-sulfuric acid method. The mean sucrose content for each entire flush was calculated.

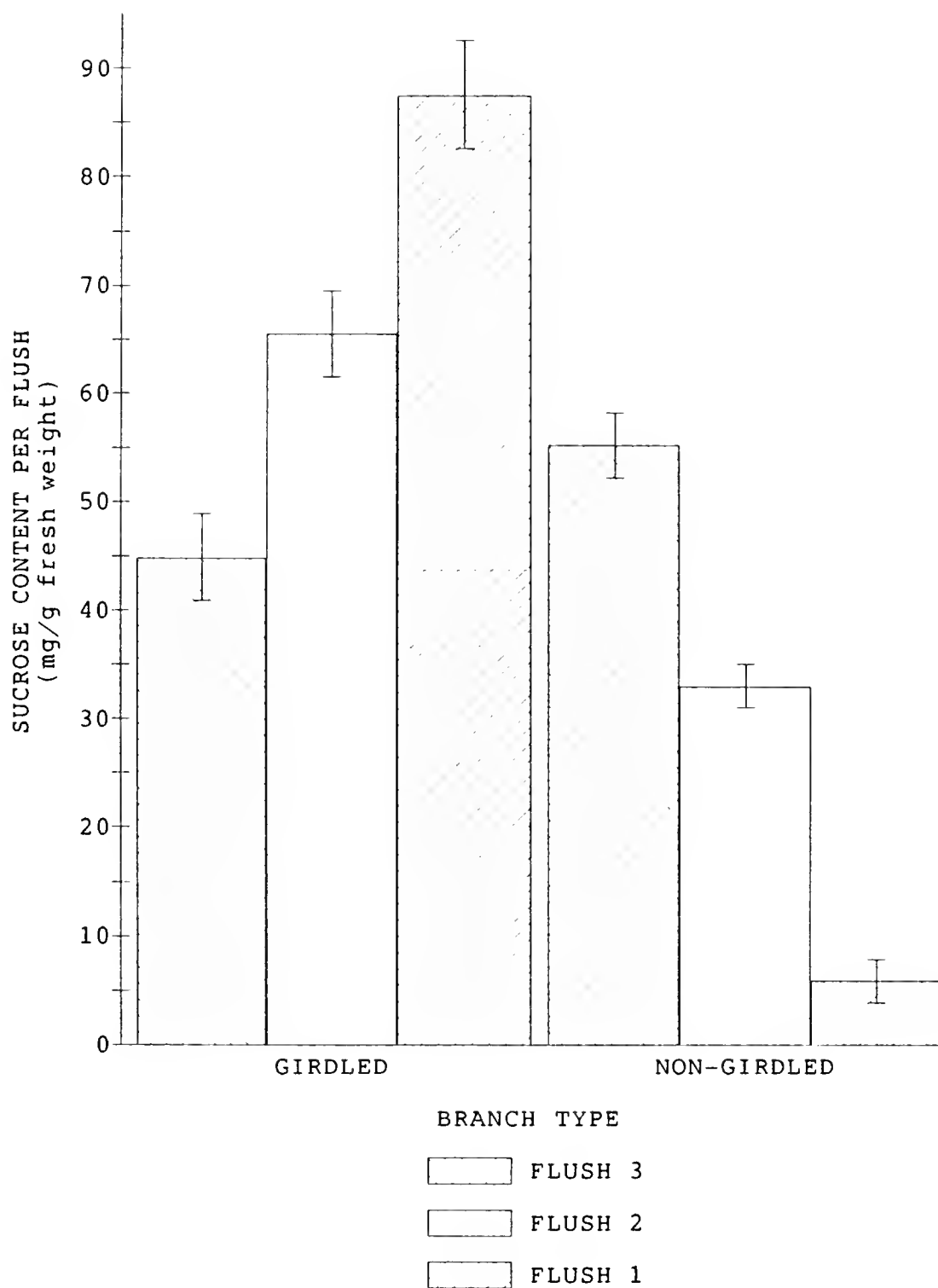


Figure 8-9. Relative Starch Content of Avocado Leaves from Three Successive Growth Flushes on Girdled vs. Non-Girdled Branches. Leaf disks (0.618 cm^2) were removed from each leaf of each flush at harvest. The samples from each flush were pooled, chopped and boiled in 85% EtOH. The residues of the ethanolic extracts were digested by the method of Housely et al. (102) and the starch quantitated by the phenol-sulfuric acid method. The mean starch content for each entire flush was calculated.

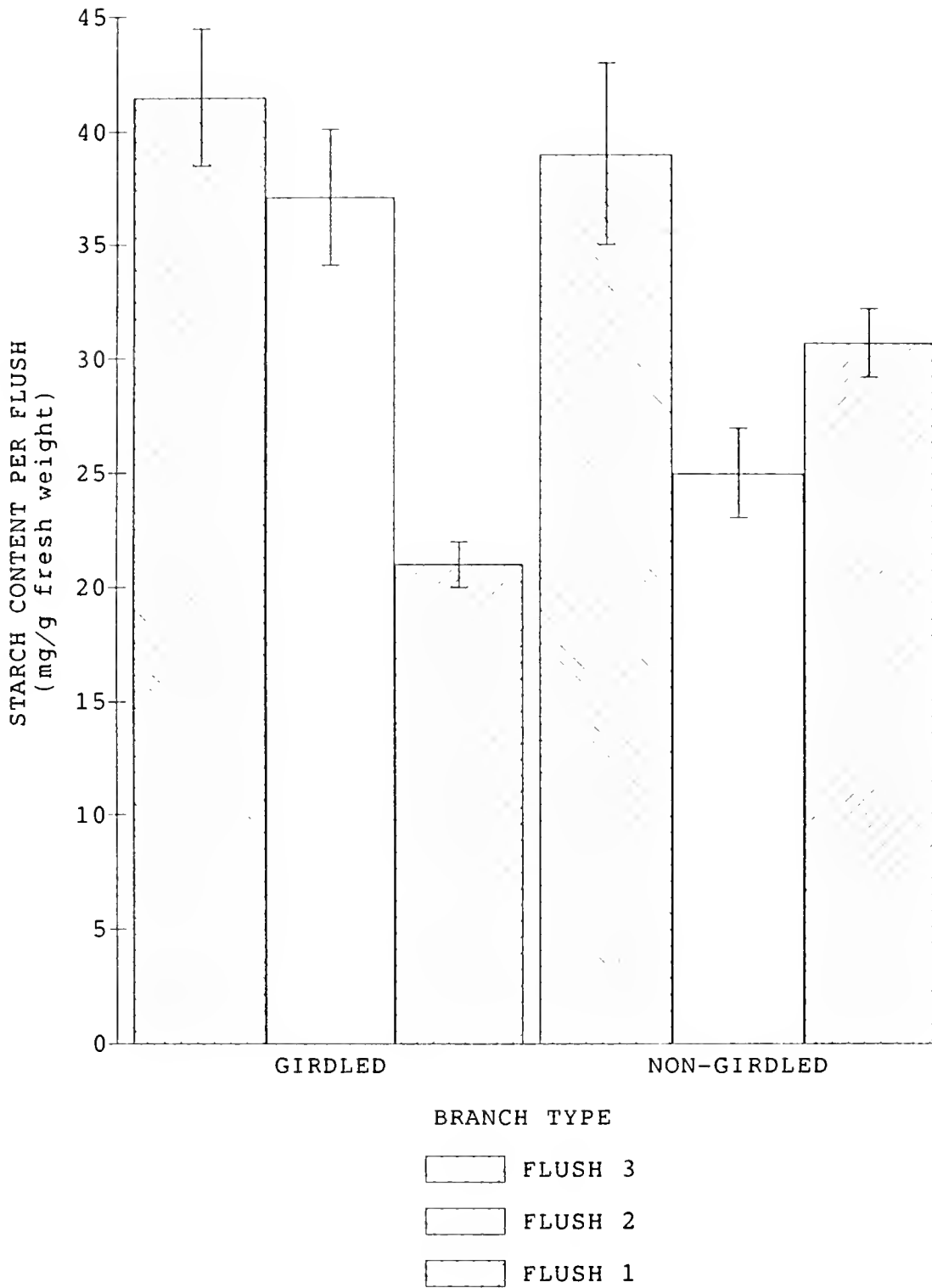
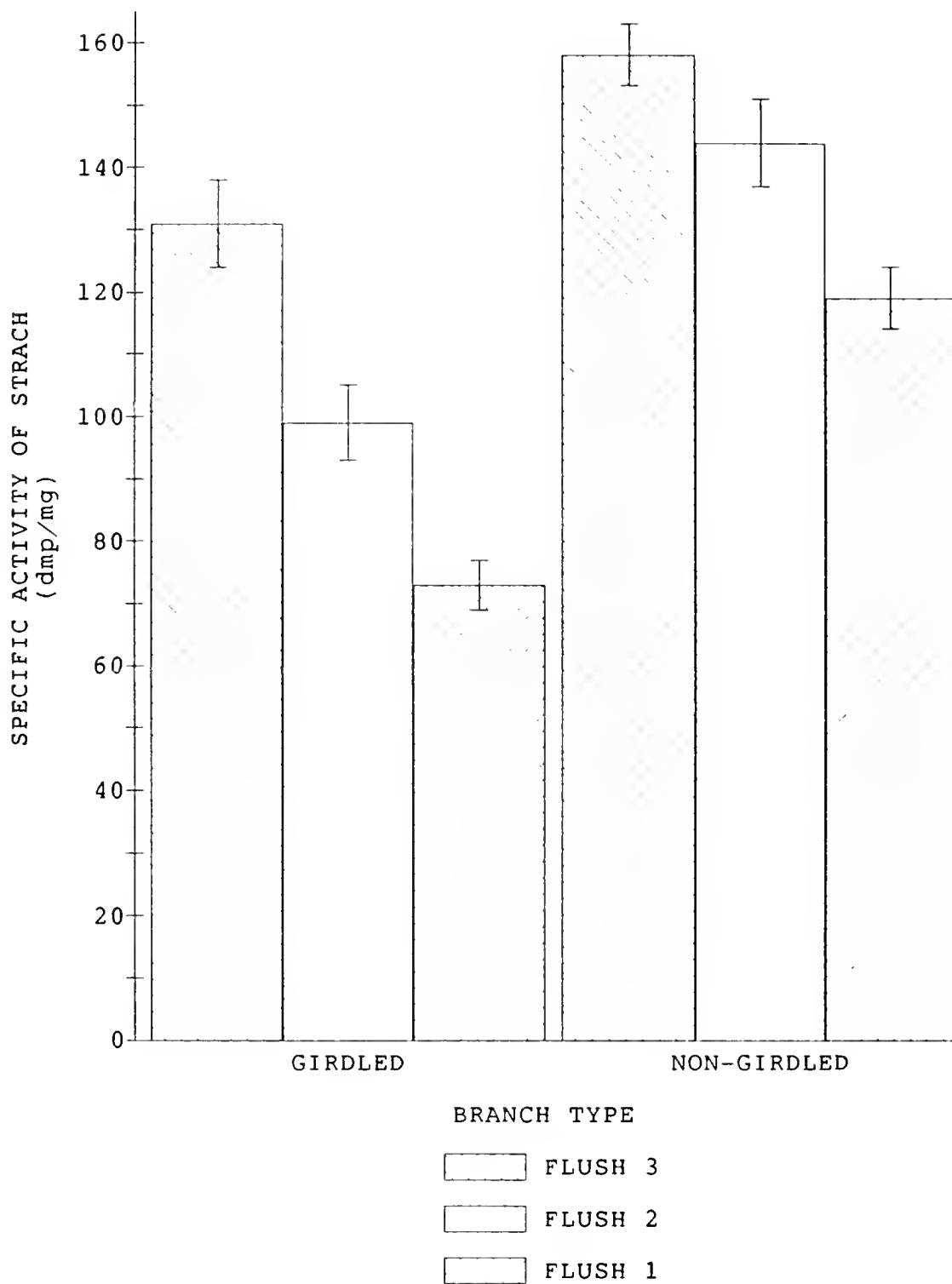


Figure 8-10. Specific Activity of Starch Extracted from Avocado Leaves of Three Successive Flushes on Girdled and Non-Girdled Branches. Leaf disks (0.618 cm^2) from each flush were pooled, chopped and boiled in 85% EtOH. The residues of the ethanolic extracts were digested by the method of Housely et al. (102) and the starch quantitated by the phenol-sulfuric acid method. Samples of known starch content were used to quantify radiolabel.



CHAPTER 9
TRANSLOCATION OF RECENT PHOTOASSIMILATES IN FRUIT-BEARING
FLUSHES IN AVOCADO: PERSEA AMERICANA MILL. CV. 'PETERSEN'

Introduction

Flowering and fruiting have frequently been examined in relation to the carbohydrate balance of the plant (76,83,118,162). The changing seasonal levels of carbohydrates in plants has often been used to explain phenomena such as alternate bearing, flower initiation, and flower and fruit abortion. In trees, the seasonal fluctuations in starch content exhibit a strong inverse correlation to the fruit load on the tree (75,76,162). It still has not been determined whether or not these seasonal starch fluctuations are the cause or coincidental with premature fruit abscission and related phenomena.

Many studies employ sink manipulation via girdling, shading or fruit removal to alter source sink relations (29,34,36,39,43,68,82,83,92,107,124,132,134,137,145,159,177,180,181,190,198,200,201). Girdling has been shown to increase floral initiation and yields (34,98,99,107,162) in several tree crops. This increase in productivity has been attributed to the accumulation of carbohydrates (134) and/or growth regulators (38,68) above the girdle. The role of fruit as a moderator of carbohydrate content has also been examined. Carbohydrates accumulate in the chloroplast when fruit are removed (93,94,99,161). Accumulation of starch may physically distort the chloroplast causing a decrease in CO₂ assimilation (140). This decrease in net CO₂ has been noted by many workers (39,43,92,93,118,140,145). Several authors

(49,50,161) contend that fruit influence CO_2 assimilation via leaf conductance and not by feedback mechanisms related to starch accumulation.

In avocado, leaf flush development and fruit development coincide for much of the season. Reproductive flushes are first evident in late January. The inflorescence emerges in a pseudoterminal position. Leaves develop at the apex as the inflorescence expands. These developing tissues are supplied assimilates by the older leaves proximal to the inflorescence (Chap. 6). The leaves distal to the inflorescence mature and become the primary supplier of photoassimilates for the developing fruit and for another apical flush (Chap. 7). This final flush begins to develop in early June. For the purpose of simplicity, the most distal flush will be designated as the third flush, the flush immediately distal to the inflorescence will be the second flush and the flush proximal to the inflorescence will be the first flush.

This and a companion chapter on the flushes of non-fruit-bearing branches (Chap. 8) are part of a series of reports which address carbohydrate involvement in the growth and abscission of avocado fruit (Chap. 2 and 3). There were several immediate goals for this research. First the assimilation, translocation, and carbohydrate content of fruit-bearing and defruited branches was measured to determine which flush was the most active in photoassimilation and translocation. Second, the starch and sucrose content of the leaves in each flush was measured so that carbohydrate pool sizes could be compared in leaves of different ages. Third, sink ratios were altered by girdling and defruiting to determine how carbohydrate metabolism was affected.

Materials and Methods

Ten-year-old, field-grown trees of Persea americana Mill. cv. 'Peterson' located at the Tropical Research and Education Center, Homestead, FL were used in this study. The most distal 3 leaf flushes on a branch were enclosed in a sealed plastic bag and labeled with 100 μCi of $^{14}\text{CO}_2$ for one a hour period between 9:00 and 11:00 a.m. $^{14}\text{CO}_2$ was generated from $\text{NaH}^{14}\text{CO}_3$ (Amersham with a specific activity of 54 mCi/mmol) using equimolar amounts of 5% lactic acid. Three branches were chosen and individually labeled as described above for each experiment. Branches were chosen from the upper, middle and lower canopy positions at each labeling. Reproductive branches each had two fruit approximately 6 cm in diameter. On defruited branches, fruit were removed either one week or two days prior to labeling. The experiment was performed 3 times.

The two distal flushes (3 and 2) represent current season's growth. The most proximal flush (flush 1) was produced during the previous season. Fruit were positioned between flush 1 and flush 2. This study was conducted late in the season when flush 3 was mature and the fruit were at their peak absolute growth rate (Chap. 2). Fruit photosynthesis was not included in this study since the magnitude of translocation of assimilate from the fruit is not significant (Chap. 5).

Translocation from the leaves was measured by taking leaf punches ($.309 \text{ cm}^2$) throughout the experimental period. The entire branch was harvested 70 hours after labeling. The leaf samples were weighed and digested with a commercial tissue solubiliser (Scintigest - Fisher

Scientific). The tissues were decolorised with 30% H_2O_2 as described by Burrell and Brunt (25). The sample vials were then filled with scintillation fluid and counted.

The metabolite pool was examined by determining the proportion of radioactivity in the acid, neutral and basic fractions of the ethanolic leaf extract. Leaf punches were taken at various times and packed in ice for transport to the laboratory. Flush samples were pooled and boiled in 80% ethanol. Samples were processed after the method of Housely et al. (102). The residue from this extraction was used to determine the starch content by the phenol-sulfuric acid method (53).

Results and Discussion

The importance of carbohydrate levels to flowering and fruiting has been reviewed by others (67,71,162). Generally, research on the carbohydrate levels in perennial tree crops has focused on seasonal fluctuations in the starch levels of the roots, trunk and branches of the tree (8,9,12,76,88,89,162). Work by Scholefield et al. (162) and Cameron and Borst (26) has shown that avocado exhibits trends similar to deciduous trees in starch content during the growing season, i.e., carbohydrate levels, specifically the starch levels decrease sharply during flowering and fruit development. A decrease in total carbohydrates has been associated with alternate bearing in "off season" avocado trees (124,162).

The effect of fruit on carbohydrate partitioning has also been studied. Altering source sink relations has effectively altered leaf and stem carbohydrate content in such diverse crops as soybean (14,23,

29,39,102,103,118,137,177,180,184), citrus (76), pepper (77,82,83), tomato (5,77,94), Easter lily (198), nectarine (38,43), and apple (87,88,89,134). In avocado fruit removal increased leaf dry mass and increased the number of starch grains in the most recent flush of leaves (161). The presence of multiple leaf flushes concurrently on a branch during fruit development provides an interesting model for carbohydrate partitioning with respect to leaf age, leaf position and fruit load.

Fruit-bearing and defruited branches had a mean of 8 leaves in the third flush, 10 in the second and 7 in the first flush. All of the leaves on the branches had approximately the same lamina length (Table 9-1). The second flush had a slightly greater area because of the greater number of leaves. In fact, the flushes of fruit-bearing and non-fruit bearing (Chap. 8), branches were similar except for a slight increase in leaf number and size of the leaves in flush 3.

Each flush of leaves on the fruit-bearing branches fixed similar proportions or about 33% of the total label fixed (Fig. 9-1). Leaves in the second flush, immediately distal to the fruit fixed slightly (37%) more label than either flush 3 (33%) or flush 1 (30%). This slight increase is probably due to the slightly larger total leaf area associated with this flush. The assimilation of label by flushes on fruit-bearing branches was much different than the age-dependent decrease in photoassimilation noted in flushes of non-fruit bearing branches (Chap. 8). Fruit sinks are known to stimulate photosynthesis (82,137,184) and delay leaf senescence (39,82,137) probably by decreasing the age-related loss of RuBPCase activity (82).

Girdling slightly decreased the ratio of assimilates fixed by flush 2. Flushes of leaves on the non-girdled branches, like their girdled counterparts fixed very similar proportions of $^{14}\text{CO}_2$ suggesting that girdling, which removed the trunk and roots as sinks, had very little effect on carbohydrate metabolism of fruit-bearing branches during the short period of this experiment. Branches defruited one week prior labeling differed markedly from the non-girdled branches in the relative fixation of label. The pattern of relative fixation in these branches was very similar to that observed in non-fruit-bearing branches (Chap. 8); the youngest flush fixed the most label (44%) and the older flushes proportionately less (flush 2-32%, flush 1-24%). Similar observations were made in experiments using soybean plants in which depodding was accompanied by decreased photosynthesis (137) and altered the partitioning of assimilates associated with senescence (39).

The flushes of non-girdled fruit-bearing branches (third, second, first) fixed 4800, 5400, 4200 dpm/mg, respectively on a dry weight basis (Fig. 9-2). The specific activity of the second flush which fixed approximately 5400 dpm/mg dry mass was only 20% higher than the value for flush one which had the lowest specific activity of the fruit-bearing flushes. Girdling and defruiting decreased the absolute specific activity of the flushes between 20 to 50%. The relative activity of flush 2 was affected the most; girdling decreased the specific activity by 65% while defruiting decreased the leaf specific activity by 55%. The trends observed for defruited and girdled fruit-bearing flushes mimiced those observed for non-fruit-bearing flushes where the youngest flush fixed the most DPM and the oldest

flush the least (Chap. 8). Although the net carbon exchange (NCE) rates of girdled and defruited plants do not change immediately after sink manipulation (67,71) it is evident from our data and others (137) that the absolute rate of photosynthesis decreased within 24 hours of sink manipulation.

The most distal flush (Fig. 9-3) had the least mass/unit area and the most proximal flush had the greatest mass per unit area. Defruiting did not affect flush dry mass; girdling did increase dry mass particularly in the oldest flush (flush 1) suggesting that the leaves on the girdled branches were acting as alternate sinks (39) for assimilates which would under non-girdled conditions be translocated to the trunk, roots or other sinks. Furthermore, the leaves of fruit-bearing branches were significantly more massive than flushes of non-fruit-bearing branches (Chap. 8)

The rate of translocation of labeled assimilates (Fig. 9-4) from the leaves on non-girdled fruit-bearing branches varied only slightly between flushes. At 24 hours post-labeling, between 39-48% of the initially fixed label remained in the leaves. Girdling decreased the rate of translocation with 48 to 62% of the label remaining in the leaves at 24 hours. Greater differences between the translocation rates of individual flushes on the girdled fruit-bearing branches were noted, for example at 19 hours flush 1 contained 62% of its initially fixed ^{14}C and flush 3 contained 48% of its initially fixed ^{14}C . The translocation rates from defruited flushes were similar to those observed in girdled fruit-bearing branches. Translocation was inhibited between 10 to 15% by girdling. This suggests that in addition to the fruit, the roots and trunk were sinks for these leaves.

Further extrapolation suggested that the leaves produced enough assimilate for both sinks, since the removal of one sink by girdling decreased photoassimilation. If photoassimilates were limiting, girdling should not alter the rate of translocation. In non-fruit-bearing branches the removal of the major sinks by girdling inhibited translocation by 50% (Chap. 8)

The relative distribution of label in the neutral (sugars), basic (amino acids), acid 1 (organic acids and sugar monophosphates), acid 2 (phosphoglyceric acid and sugar diphosphates) fractions was not significantly different between fruit-bearing and non-fruit-bearing branches or the flushes on these branches (Table 9-2); suggesting, that recent photoassimilates were incorporated the into various soluble pools in the same proportions in both branch types. Even though relative pool sizes were the same, the absolute pool size of neutral sugars was larger in fruit-bearing branches. Similar observations have been made in citrus (72) and apple (84). The absolute quantity of ethanol soluble sugars (gm sucrose eq./gm fresh weight) (Fig. 9-5) was higher in leaves of girdled branches of both non-fruit bearing and fruit-bearing branches. However within a given treatment the fruit-bearing branches had the higher sucrose content. The most recently formed flush on the non-girdled branch, flush 3, had the highest sucrose content on a fresh weight basis (100 mg/gm fresh weight). Flushes 2 and 1 contained similar amounts of sucrose (approximately 50 mg sucrose/gm fresh tissue). The sucrose content for the three girdled fruit-bearing flushes ranged between 110-120 mg sucrose/gm fresh tissue. Defruited branches contained less than 50 mg sucrose/gm fresh tissue in the two most recent flushes.

The specific activity (Fig. 9-6) of sucrose extracted from the older flushes (1 and 2) was elevated in the girdled (3,000 and 5,000) and defruited (4,000 and 5,000) flushes compared to their non-girdled counterparts (2,500 and 4,000). The specific activity of the sucrose in the most distal flush was greater in defruited (15,000) and girdled (10,000) branches than in non-girdled fruit-bearing branches (7,000). Although the distal flushes of girdled and non-girdled fruit-bearing and defruited flushes had similar dry mass, there was an apparent difference in how recent photoassimilates were distributed to the carbohydrate pools.

Girdling and defruiting produced a highly labeled sucrose pool in the most recent flush of the branches. Girdling and defruiting decreased the sink demand on the flush. How can the higher specific activity of sucrose in the girdled fruit-bearing and defruited flushes be explained? One explanation involves the allocation of sucrose to a non-transport pool. Mature leaves exhibit compartmentation of sucrose within their cells (72,73). Two pools of sucrose have been suggested (72,73) one for export from the mesophyll into the apoplast for phloem loading and the other non-transport pool which is mobilized on demand. In this case, a portion of the labeled sucrose was present and maintained in a non-transport pool while the remainder was translocated or converted to starch. The size of this non-transport pool may correlate to sink demand (73). However, over the short period of this experiment the pool sizes may not have compensated for the change in sink demand, therefore the specific activity of sucrose in flush 3 was greater than expected.

Increased translocation rates have been associated with increased sucrose phosphate synthase (SPS) activity (153) which in turn correlates to increased sink demand. Leaves on fruit-bearing branches may exhibit increased SPS activity resulting in an increase in sucrose labeling and a decrease in starch accumulation (101,171). In avocado, sink demand apparently did increase sucrose labeling. Leaves on fruit-bearing flushes maintained a highly-labeled, soluble sucrose pool.

The leaves of the most distal flush on the non-girdled branch contained the highest proportion of starch (54%) (Fig. 9-7). Generally, the flushes of girdled branches had higher percentages of starch than their non-girdled counterparts (28% girdled vs. 17% non-girdled). Defruiting depressed the starch content of the distal flush (54% vs. 34%, non-girdled vs defruited) yet caused an increase in the starch content of the oldest most acropetal flush (34 vs 44%, non-girdled vs defruited). The flushes which flank the fruit (flush 1 and 2) had the lowest starch content. Flush 2 contained the lowest percentage of starch on both non-girdled and defruited branches. Other work done in this laboratory has shown that the majority of assimilate translocated from the proximal flush travels basipetally at this stage of leaf development (Chap. 6). Flush 2, which was distal to the fruit provided assimilate to both the fruit and to the developing apex. The depression in the starch content probably reflected the mobilization of recent reserves to the developing sinks (flush 3 and the fruit).

The specific activities (Fig. 9-8) of starch extracted from girdled fruit-bearing branches or defruited branches were similar. The highest specific activity was found in starch extracted from the

second flushes of defruited and girdled fruit-bearing branches. So although the fruit-bearing flushes fixed similar amounts of $^{14}\text{CO}_2$, the second fruit-bearing flush directed more recently fixed carbon into gluconeogenesis when sink strength decreased. The first and third flushes had similar values which were about 60% that of flush 2. The starch extracted from leaves on non-girdled fruit-bearing flushes had the highest specific activity. Unlike the pattern observed in the girdled and defruited branches, the specific activity of the starch extracted from leaves on non-girdled fruit-bearing flushes increased with the age of the flush.

This paper and its companion paper (Chap. 8) have shown that fruit-bearing and non-fruit-bearing branches were very different in their abilities to assimilate and translocate recent photoassimilates. Leaves of similar age on fruit-bearing branches were more photosynthetically competent than their non-fruit-bearing counterparts. All of the flushes on fruit-bearing branches fixed similar proportions of $^{14}\text{CO}_2$ and translocated their recently fixed photoassimilates more rapidly than non-fruit-bearing branches. Unlike the pattern observed in fruit-bearing branches, the fixation and translocation of radiolabeled assimilates by non-fruit-bearing branches was age dependent, with the youngest flushes being the most competent (Chap. 8). Non-fruit-bearing and fruit-bearing branches also differed in dry mass, soluble sugar content, and starch content. The pool sizes of the neutral fraction (sugars) was much larger for fruit-bearing flushes, although relative pool sizes (neutral, basic, acid 1, acid 2) were the same for all flushes.

The values for the relative specific activity of starch extracted from different flushes on non-fruit-bearing branches were similar suggesting that recent photoassimilates were similarly allocated to this pool in all flushes; however, fruit-bearing flushes allocated recent photoassimilates to the starch and soluble sugar pools differently; the second flush directed more of its recent photoassimilates into starch biosynthesis and less into soluble sugar pools while the most distal flush directed more assimilate to its soluble sugar pools than to starch synthesis. The translocation and fixation rates for flushes on the fruit-bearing flushes were nearly the same.

Altering the source to sink ratio by girdling had its most striking effect on translocation rates. In non-fruit-bearing branches girdling decreased the movement of recent photoassimilates by approximately 50%. Girdling in this case removed the primary sink. However, in fruit-bearing branches girdling decreased the rate of translocation by only 15%. This indicated that the fruit was the major sink, but it also suggested that enough assimilate was produced to supply both the fruit and an alternate sink, the trunk and roots. If this were not the case, and only enough assimilate were produced to supply the fruit then no decrease in the translocation rate would be expected.

Table 9-1. Summary of Avocado Branch Characteristics.

Each branch was dissected at harvest and the leaves were counted and measured. Lamina length equals that of the central vein in the blade portion of the leaf (petiole excluded). Area was determined using a leaf area meter.

	Flush #	# of Leaves	Lamina Length(mm)	Area (cm ²)	# of Branches
Fruit-bearing	3	7.0 \pm 4.1	77.0 \pm 12	157 \pm 25.3	8
	2	10.4 \pm 4.0	79.7 \pm 8.0	264 \pm 48.4	
	1	7.0 \pm 2.0	78.2 \pm 9.1	194 \pm 64.2	
Defruited	3	9.0 \pm 3.3	78.4 \pm 6.2	175 \pm 303	
	2	10.0 \pm 4.4	79.4 \pm 3.5	214 \pm 73	
	1	7.0 \pm 3.1	81.7 \pm 5.8	206 \pm 34	

Table 9-2. Time Course of Changes in the Relative Pool Sizes of Soluble Radiolabeled Compounds From Avocado Leaves.

Samples were taken from leaves at various time points and processed by the method of Housely et al. (102). The neutral fraction represents the sugars; the basic fraction consists of amino acids; organic acids and sugar monophosphates are included in the acid 1 fraction; phosphoglyceric acid and sugar diphosphates are included in the acid 2 fraction. These results are presented as percentages of the total radioactivity recovered from the columns. Time is given in hours post-labeling.

Time (hr post-labeling)	Neutral (% of total radiolabel recovered from column)	Basic	Acid 1	Acid 2
0	90.4 \pm 2.5	5.3 \pm 3.8	3.6 \pm 3.0	0.5 \pm 0.3
24	88.8 \pm 5.0	7.1 \pm 4.9	2.8 \pm 1.7	1.2 \pm 0.9
52	72.5 \pm 7.5	5.2 \pm 2.7	6.8 \pm 5.7	5.2 \pm 4.7
78	66.7 \pm 0.7	19.4 \pm 5.5	8.2 \pm 3.9	5.8 \pm 2.2

Figure 9-1. Relative Distribution of ^{14}C -Labeled Assimilates in Three Successive Avocado Leaf Flushes on Fruit-Bearing and Defruited Branches. Leaf samples (0.618 cm^2) were taken from each leaf of each flush on both girdled and non-girdled, fruit-bearing and defruited branches immediately after the 1 hr labeling period. These samples were dried, weighed, digested and the radiolabel quantified as described in the Material and Methods. At the end of the experiment, branches were harvested and leaf area was measured. Leaf area and label incorporation after 1 hr exposure to $^{14}\text{CO}_2$ were used to calculate the proportion of label incorporated by each of ²three consecutive, girdled or non-girdled flushes. The fruit were removed from defruited branches one week prior to labeling. Branches were approximately 70-80 cm in total length and 1 to 1.5 cm in diameter. Branches were girdled proximal to the oldest flush, adjacent to the main scaffolding branch. Three branches were chosen, 1 each from the lower, middle and upper canopy positions. The experiment was repeated four times.

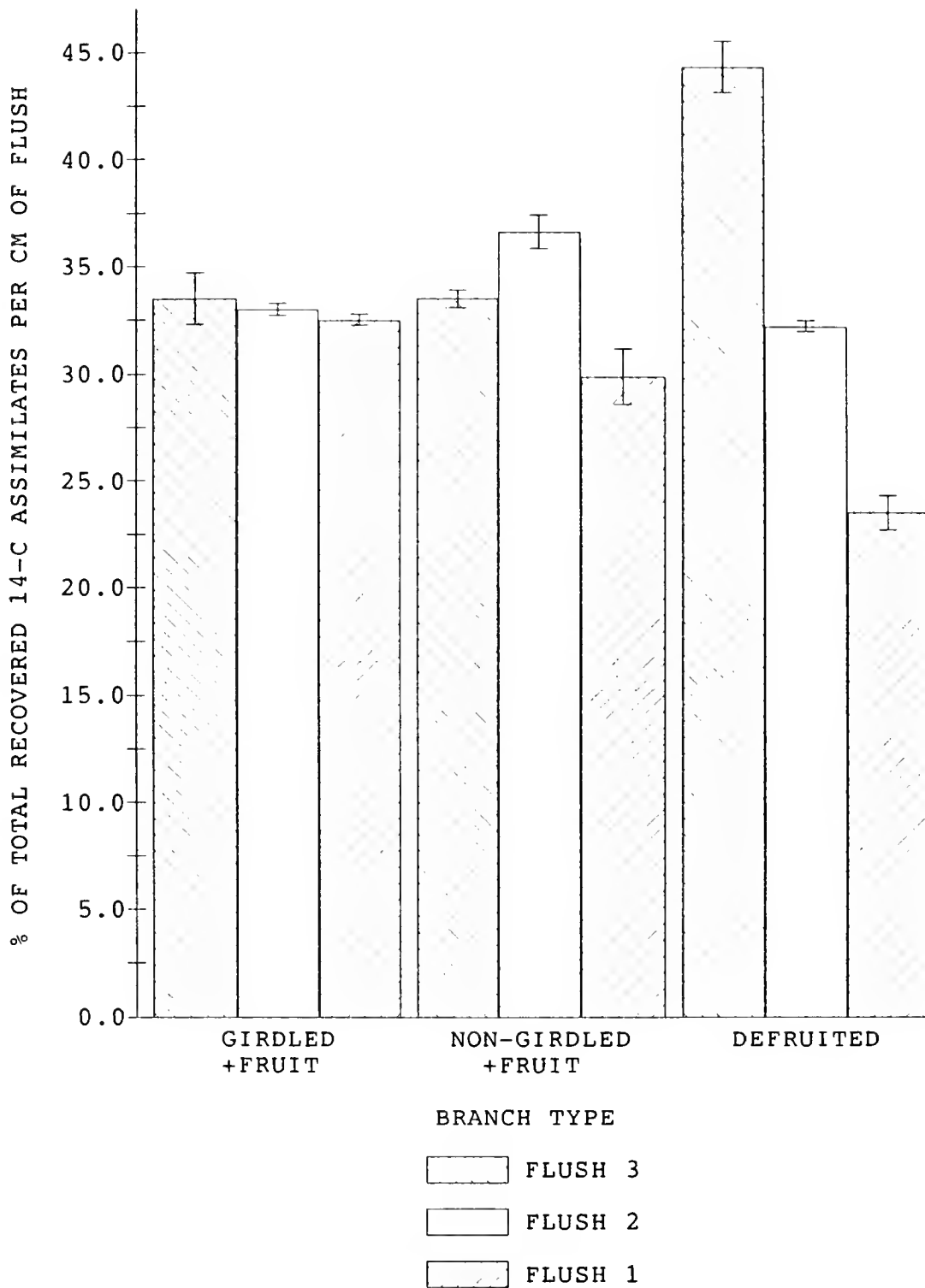


Figure 9-2. Specific Activity of Avocado Leaf Tissue from Three Successive Leaf Flushes on Fruit-Bearing and Defruited Branches. Leaf samples were taken from each leaf of each flush on both girdled and non-girdled; fruit-bearing and defruited branches immediately after the 1 hr labeling period. These samples were dried, weighed, digested and the radiolabel quantitated as described in the Materials and Methods. Leaf disk (0.618 cm^2) dry mass and label incorporation after 1 hr exposure to $^{14}\text{CO}_2$ of all the leaves in the flush were used to calculate the mean incorporation of label by each of three consecutive, girdled or non-girdled flushes. The fruit were removed from defruited branches one week prior to labeling. Branches were approximately 70-80 cm in total length and 1 to 1.5 cm in diameter. Branches were girdled proximal to the oldest flush, adjacent to the main scaffolding branch. Three branches were chosen, 1 each from the lower, middle and upper canopy positions. The experiment was repeated four times.

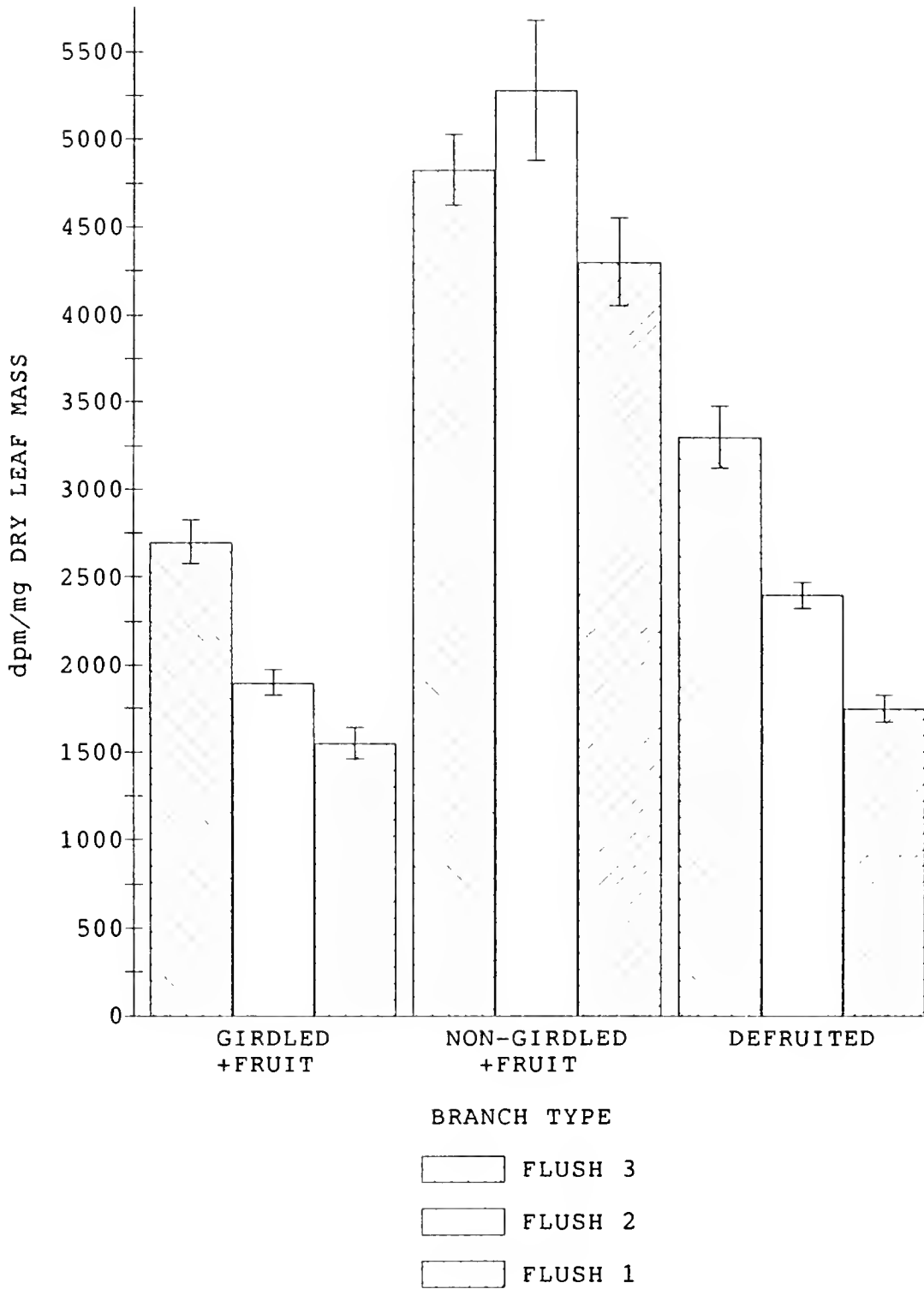


Figure 9-3. Specific Leaf Weight of Avocado Leaves from Three Successive Growth Flushes on Girdled and Non-Girdled; Fruit-Bearing and Defruited Branches. Leaf disks (0.618 cm^2) were removed from each leaf of each flush on both girdled and non-girdled branches. The leaf disks were dried and weighed as described in the Materials and Methods. The fruit were removed from defruited branches one week prior to labeling. Each flush bore at least 10 leaves and each leaf was sampled at least 4 times during the experiment.

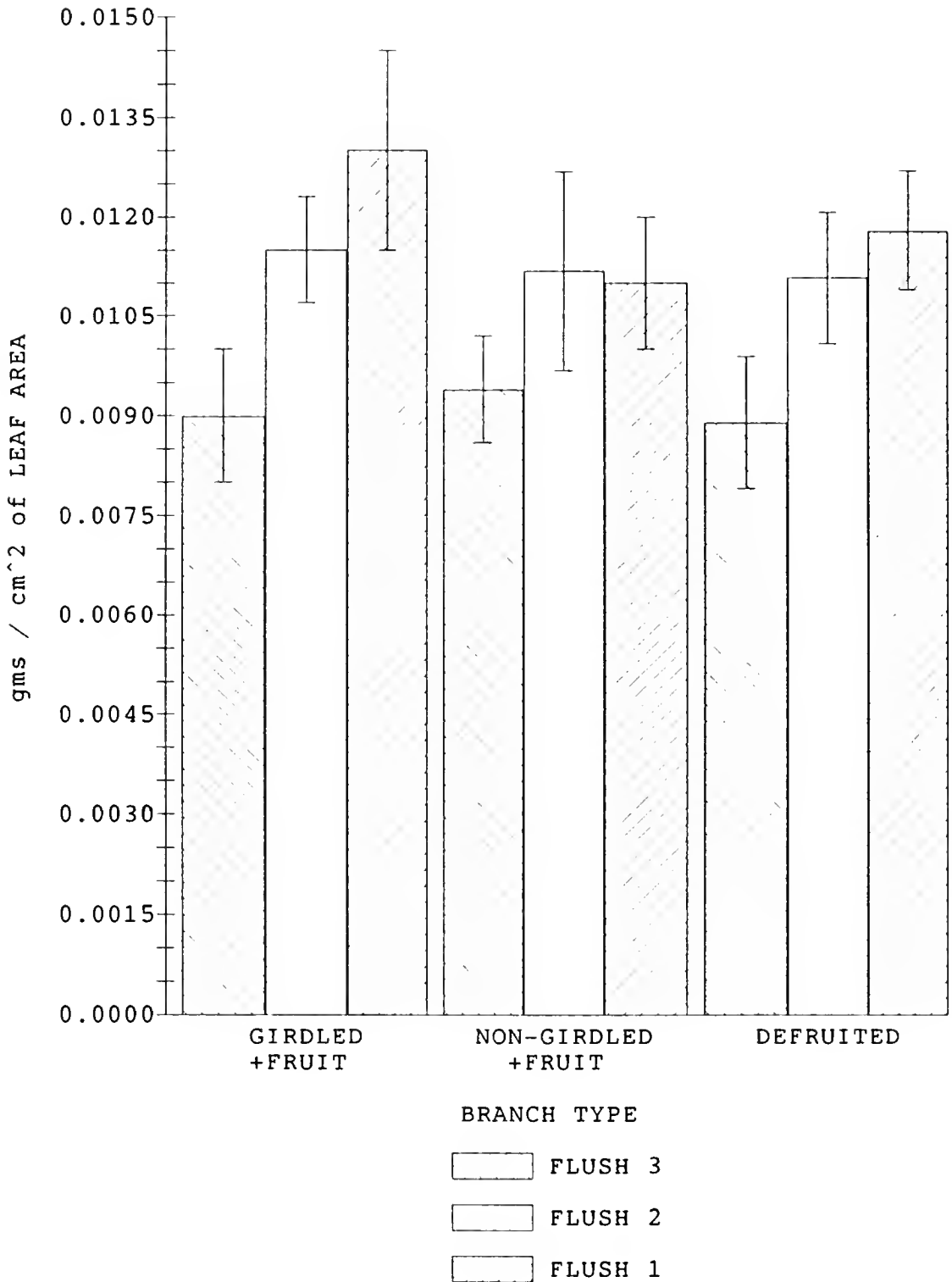


Figure 9-4. Export of Labeled Assimilates from Leaves of Three Successive Growth Flushes on Girdled and Non-Girdled Fruit-Bearing and Defruited Avocado Branches. Leaf disks taken throughout the experimental period were dried, weighed and digested as described in the Materials and Methods. The fruit were removed from defruited branches one week prior to labeling. Translocation was defined as the difference between the amount of radiolabel present immediately after a 1 hr pulse of $^{14}\text{CO}_2$ and the given time point during the 75 hr chase.

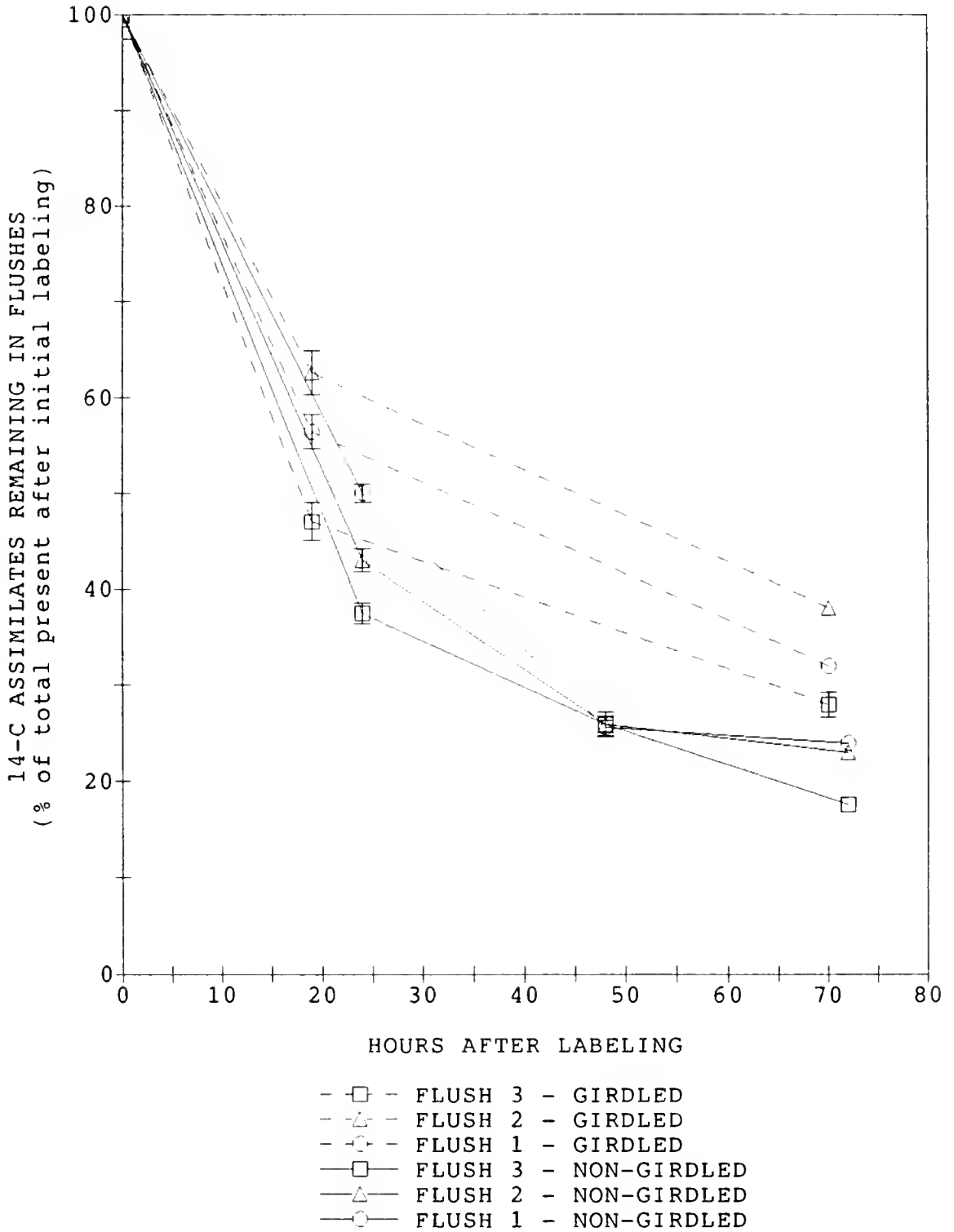


Figure 9-5. Sucrose Content per Unit Fresh Weight of Avocado Leaves from Three Successive Growth Flushes on Girdled and Non-Girdled Fruit-Bearing and Defruited Branches. Leaf disks (0.618 cm^2) were removed from each leaf of each flush at harvest. The samples from each flush were pooled, chopped and boiled in 85% EtOH. The ethanolic extracts were concentrated and the sugar content was measured by the phenol-sulfuric acid method. The fruit were removed from defruited branches one week prior to labeling.

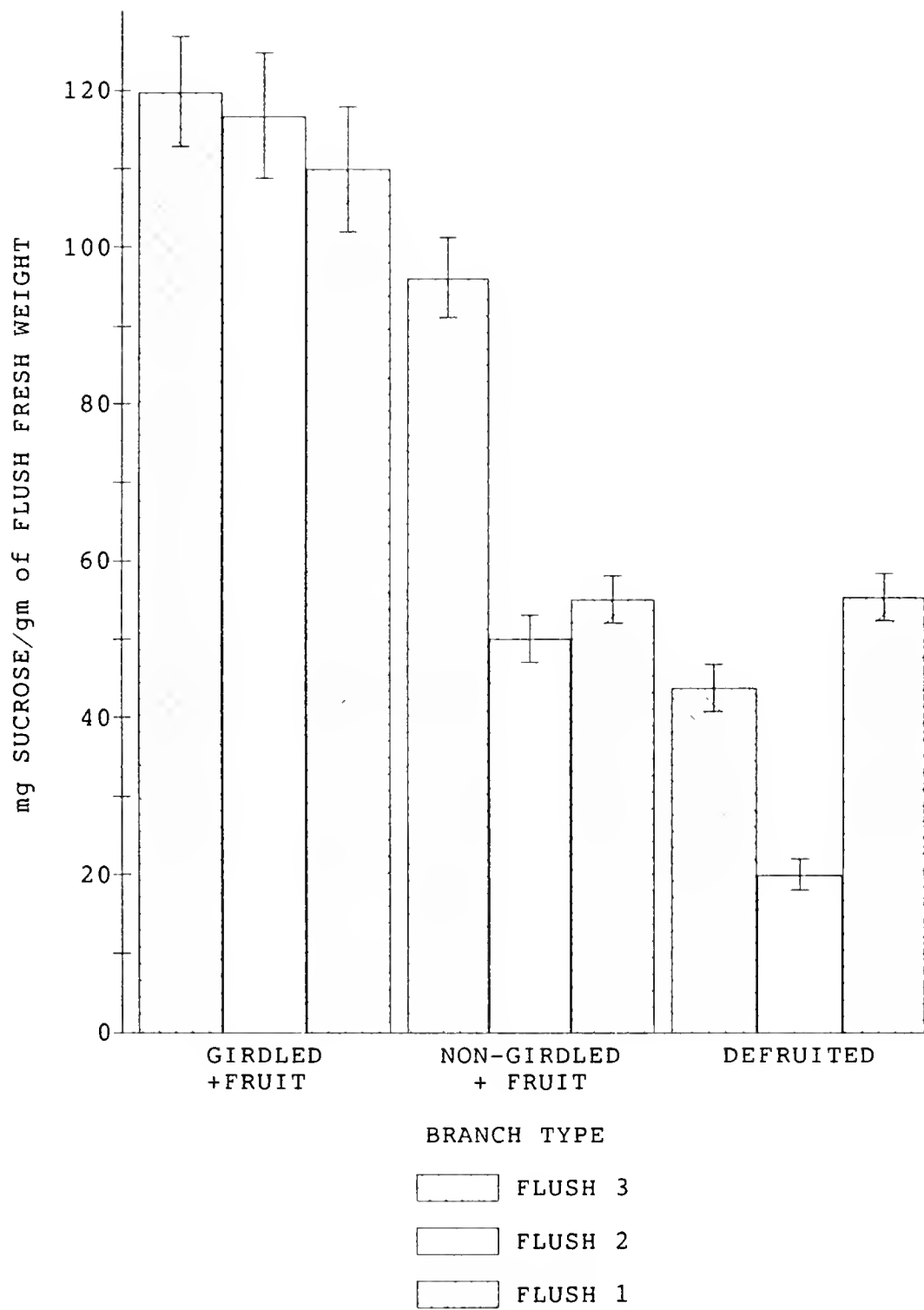


Figure 9-6. Specific Activity of Sucrose Extract from Avocado Leaves of Three Successive Flushes on Girdled and Non-Girdled Fruit-Bearing and Defruited Branches. At harvest, leaf disks (0.618 cm^2) were removed from each leaf of each flush. The samples from each flush were pooled, chopped and boiled in 85% EtOH. The ethanolic extracts were concentrated. Sugar content was quantitated by the phenol-sulfuric acid method. Samples of known sucrose concentration were decolorized for scintillation counting. The fruit were removed from defruited branches one week prior to labeling.

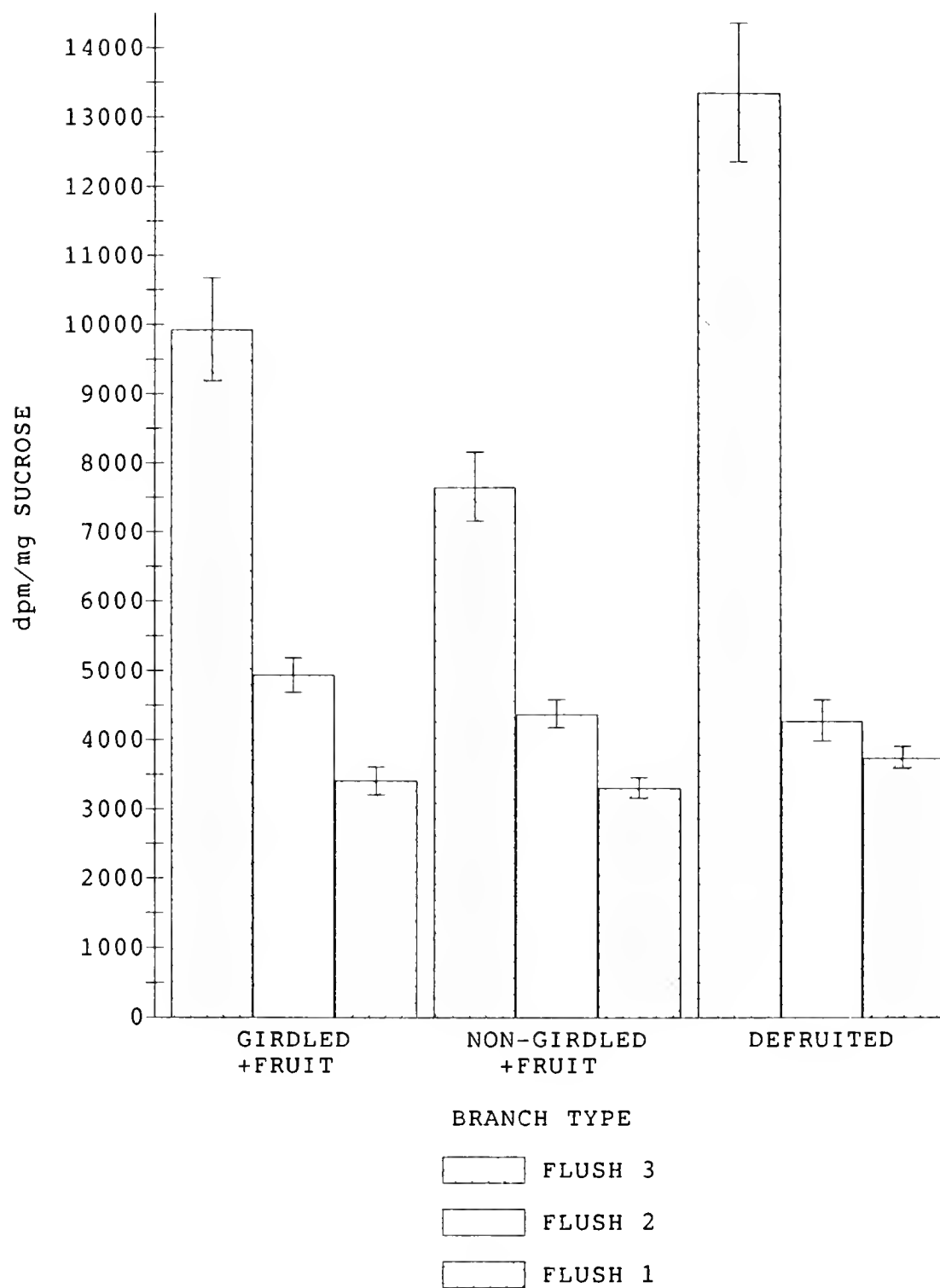


Figure 9-7. Relative Starch Content of Avocado Leaves from Three Successive Growth Flushes on Girdled vs. Non-Girdled Fruit-Bearing and Defruited Branches. Leaf disks (0.618 cm^2) were removed from each leaf of each flush at harvest. The samples from each flush were pooled, chopped and boiled in 85% EtOH. The residues of the ethanolic extracts were digested by the method of Housely et al. (102) and the starch quantitated by the phenol-sulfuric acid method. The mean starch content for each entire flush was calculated. The fruit were removed from defruited branches one week prior to labeling.

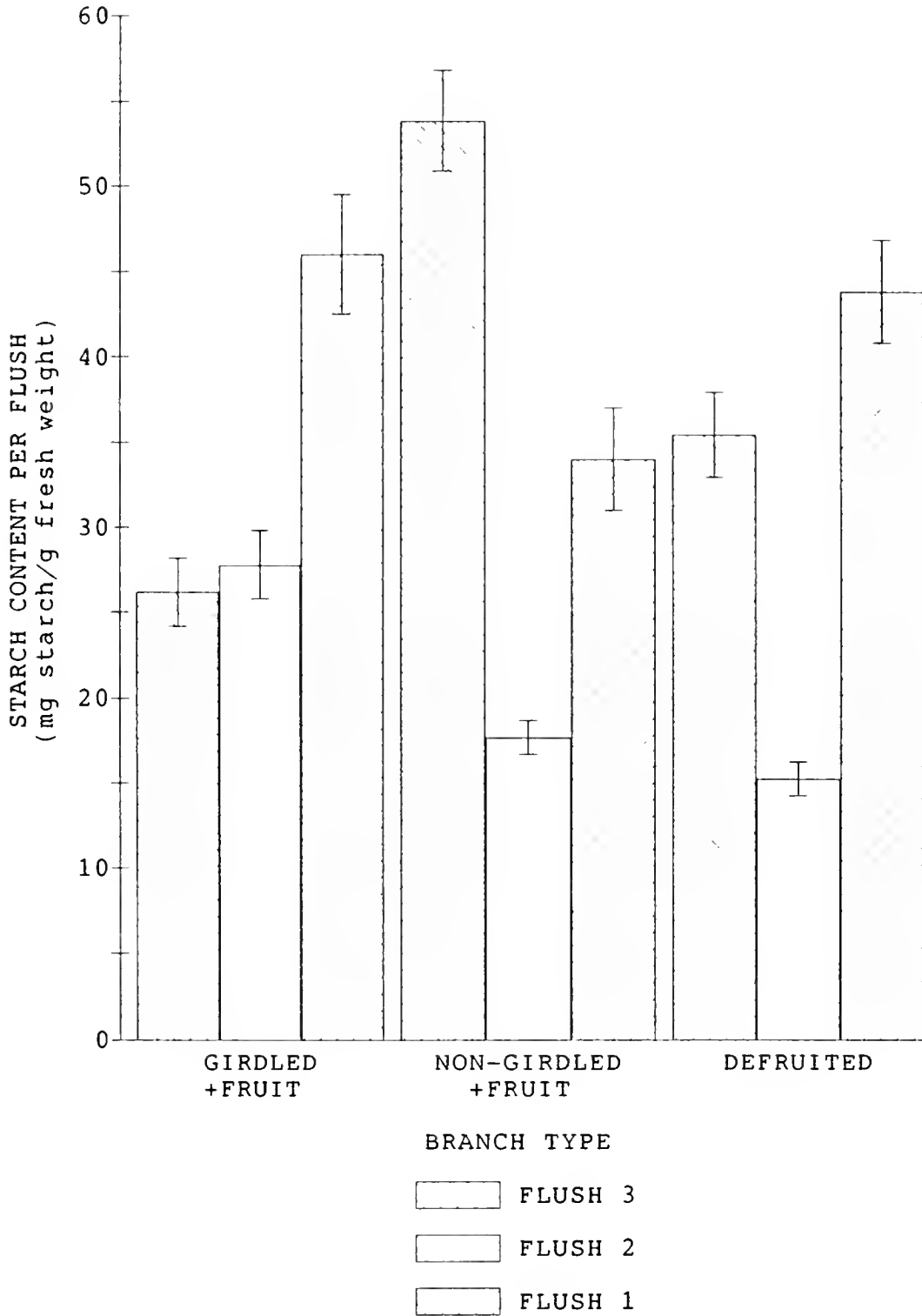
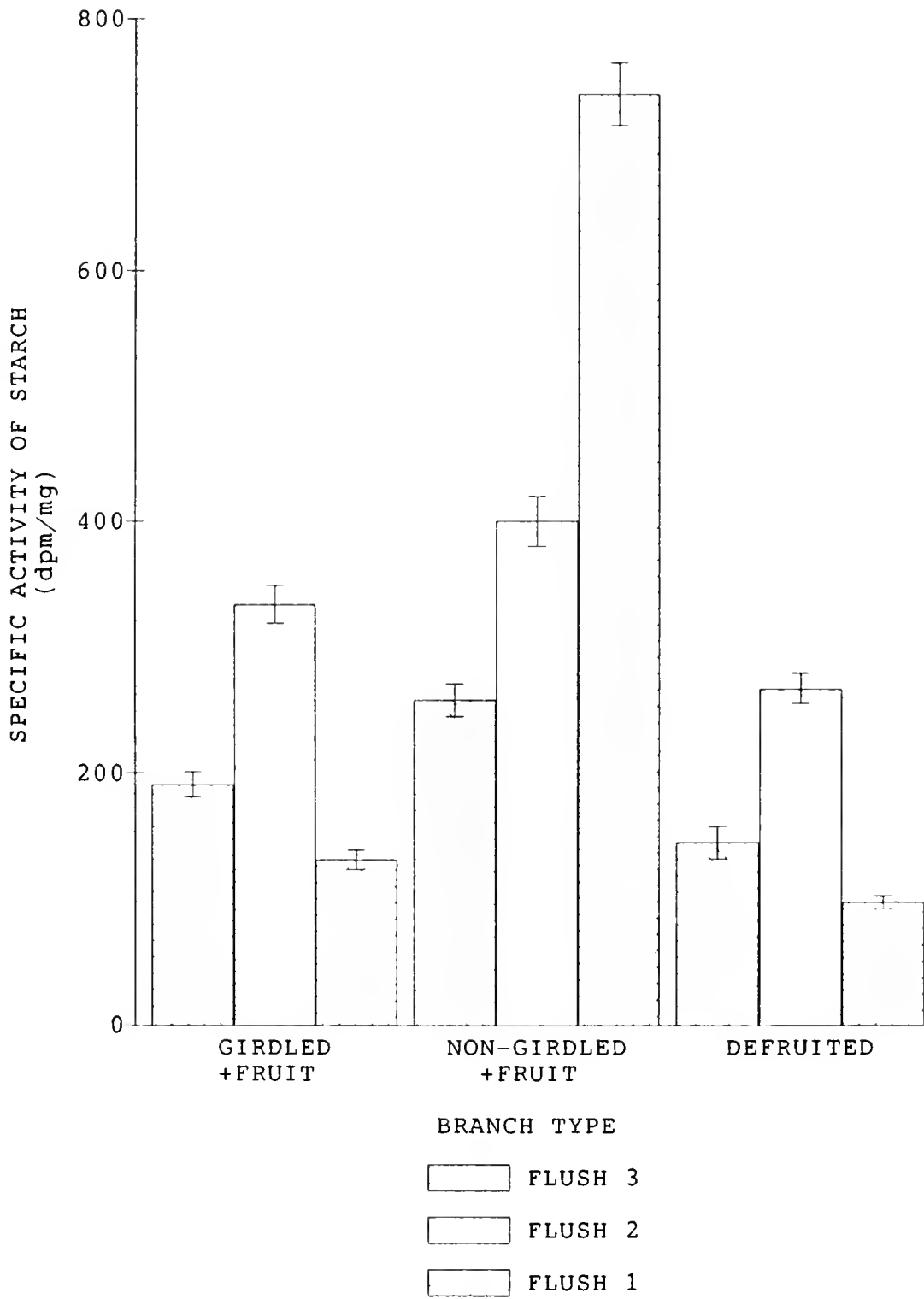


Figure 9-8. Specific Activity of Starch Extracted from Avocado Leaves of Three Successive Flushes on Girdled and Non-Girdled Fruit-Bearing and Defruited Branches. Leaf disks (0.618 cm^2) from each flush were pooled, chopped and boiled in 85% EtOH. The residues of the ethanolic extracts were digested by the method of Housely et al. (102) and the starch quantitated by the phenol sulfuric acid method. Samples of known starch content were used to quantify radiolabel. The fruit were removed from defruited branches one week prior to labeling.



CHAPTER 10

SUMMARY

Approximately 20% of flowers produced by avocado trees will form fruit but only 1 to 7% of the fruit will remain at harvest. Competition between developing reproductive structures and leaves for limiting carbohydrates may cause the abscission of avocado flowers and fruit because, they form in a pseudoterminal position and develop concurrently with new apical leaves. This study was undertaken to determine if the availability of local photosynthates affected fruit abscission. Several lines of inquiry were made to study this hypothesis, including the identification of the phloem mobile sugars, measurement of fruit growth and yield, examination of assimilate movement into abscising and non-abscising fruit, and studies on the movement of radiolabel on fruit-bearing and non-fruit-bearing branches.

The major sugar components in alcoholic extracts and phloem exudates from representatives of the three races of avocado (Persea americana Mill.) were analyzed using thin layer chromatography and differential staining procedures. Sucrose and mannoheptulose were the predominant sugars found in leaf extracts and phloem exudates. Leaves fed $^{14}\text{CO}_2$ produced radiolabeled sucrose exclusively as the labeled translocated sugar for up to 24 hours in all three races of avocado during the summer months (May to August). During the summer months radiolabeled sucrose was observed to move from the lamina to the major veins within one hour after labeling. During the winter months (Nov.

to Jan.) $^{14}\text{CO}_2$ incorporated during photosynthesis was recovered in both sucrose and mannoheptulose. Although mannoheptulose was present in avocado leaf extracts and leaf phloem exudates in quantities similar to sucrose and was labeled during the winter months, its role is presently unclear.

The diameters of abscising and persisting avocado fruits were measured every other day in order to compare growth and abscission kinetics. Abscising and persisting avocado fruit exhibited similar growth kinetics until 4 to 5 days before fruit separation, at which time the rate of fruit expansion decreased in abscising fruit. The greatest relative growth rate ($0.18921 \text{ cm}^3 \text{ cm}^{-3} \text{ d}^{-1}$) occurred immediately post-anthesis. Maximum absolute growth rate values ($2.2897 \text{ cm}^3 \text{ d}^{-1}$) occurred at approximately 110 days post-anthesis. Eighty percent of the measured fruit abscised during the experiment. The number of fruit on a branch did not affect the fruit's growth rate or probability of abscission.

Fruit growth and the distribution of radiolabeled assimilates were studied in abscising and persisting fruit. Abscising fruit accumulated significantly greater proportions of radiolabel in the seed coat when compared to persisting fruit. Persisting fruit increased the longevity of punctured fruit on the same inflorescence and extended the period in which assimilate distribution was altered. Puncturing the seed coat caused a transient increase in its sink strength. Restriction of assimilate movement in punctured fruit was evident within 24 hours and complete within 72 hours. Fruit separation occurred 24 to 48 hours later.

The ability of avocado reproductive structures to assimilate photosynthetic products either directly or via transport from source leaves was evaluated. The accumulation of labeled assimilates in the reproductive organs and tissues was estimated after flowers, fruits and source leaves were fed $^{14}\text{CO}_2$. The mean sink strength (total dpm) of flowers and flower buds was less than half that of fruits. The sink intensity (dpm mg^{-1} dry mass) of the flowers and flower buds was 20 times that of the fruit. Developing fruit fixed $^{14}\text{CO}_2$ in proportion to their dry mass. Avocado leaves fixed the majority (96%) of the available labeled CO_2 while the fruit and flowers fixed only 4% of the label. Although some label fixed by the fruits was translocated to other tissues, the majority (97-99%) of the radiolabel remained in the fruit. CO_2 fixation by the flowers and fruits of avocado did not contribute substantial photoassimilate to fruit growth.

Inflorescences of avocado form in a pseudoterminal position and develop concurrently with new, apical (distal) leaves. The potential 'competition' between developing leaves, flowers and fruit for limiting amounts of available assimilate as a cause of flower and fruitlet abscission was studied by examining the movement of ^{14}C -labeled photoassimilates from mature leaves located proximal to the inflorescence at all stages of inflorescence and distal leaf development. Assimilates moving from the proximal leaves were equally divided between acropetal and basipetal sinks at the earliest stage of distal leaf development (midvein length <20 mm). As the inflorescences began to bear developing fruitlets (<1 cm diameter) and distal leaves continued to develop, 5-times more label moved acropetally to supply

the developing vegetative and fruit mass than basipetally from the proximal leaves. In the final stages of distal leaf development the percentage of label going from the proximal leaves to distal reproductive and vegetative organs decreased while the percentage traveling basipetally increased. At all periods of development, the sink strength of flowers, fruits and leaves as determined by accumulated labeled assimilate was proportional to their dry mass. Thus, on a per unit mass basis developing fruitlets and distal leaves were equally effective in mobilizing recent photoassimilates. The availability of recent photoassimilates from local sources did not appear limiting since radiolabel was recovered acropetally and basipetally to fruit and does not explain the high abscission rate observed in avocado.

The leaves developing distal to the avocado inflorescence initially represent a potentially competing sink and eventually at maturation a source of assimilate for the developing fruits. The partitioning of recent photoassimilates during development of distal leaves was studied in relation to the leaf's sink/source transition. Avocado leaves reached their peak import rate at 22% final midvein length. Import decreased and was negligible at 37% final midvein length. Export of newly fixed assimilates from the developing leaves began at 20% final midvein length and peaked at 60% final midvein length. Fruits having the same orthostichy as the labeled source leaf received up to 95% of the radioactivity recovered from all of the fruit sampled from the same branch. Distribution of label to fruit with the same aligned orthostichy was a function of fruit mass and distance from the source leaf. The apparent precocious maturation of the leaves distal

to the inflorescence, as indicated by assimilate export, suggested that the developing leaves were autonomous earlier than previously thought. Subsequently, the period of possible leaf/fruit competition would be diminished.

Tropical evergreen trees frequently undergo several growth flushes each year. Persea americana cv Mill. growing in Florida commonly undergo 2-3 growth flushes a year; one prior to floral initiation, a second immediately post-anthesis, and a third approximately 8 weeks post-anthesis. Fruit-bearing branches of avocado consisting of 3 flushes were fed $^{14}\text{CO}_2$ and then characterized for translocation rates and carbohydrate content. The leaves on non-girdled reproductive and defruited branches translocated radiolabeled assimilate at similar rates. Girdling decreased translocation of radiolabeled assimilates from flushes by 15-20%. The leaf specific activity was 2-times greater in the most recent flush than in the oldest flush. Leaf dry mass increased in parallel with increased photoassimilation by leaves on fruit-bearing branches. Sucrose content was higher in girdled than non-girdled branches. Photoassimilation and assimilate movement within mature non-fruit-bearing flushes was also examined. Non-fruit bearing branches of avocado consisting of 3 recent flushes were fed $^{14}\text{CO}_2$ and then characterized for translocation rates and carbohydrate content. Leaf specific activity was 3-times greater in the most recent flush than in the leaves of the oldest flush. The youngest flush fixed greater than 50% of the total available label, while the oldest flush fixed less than 20% of the available label. Relative pool sizes were identical in all three flushes although the absolute amount of soluble sugars was greatest in the youngest flush. Leaf dry mass was inversely

proportional to photoassimilation. Girdling decreased the assimilation of label between 20 to 50% and the translocation of radiolabeled assimilates from the leaves by approximately 50%. Sucrose and starch content was greater in the leaves of girdled than non-girdled branches. Flush age did affect photoassimilation and translocation in non-fruit-bearing branches of avocado. The partitioning of recent photoassimilates between soluble and insoluble carbohydrate pools in avocado varied between flushes and was dependent on fruit-bearing status.

Carbohydrate limitation did not appear to influence avocado fruit abscission. Approximately 80% of the measured fruit in this study abscised regardless of fruit load on the branch. Furthermore, during the peak period of abscission, assimilates from the proximal source leaves were supplying assimilates to both the acropetal sinks (fruit and developing leaves) and the basipetal sinks (roots). Avocado leaves exported assimilate at an early stage of development suggesting that the period of competition between fruit and developing leaves was shorter than anticipated. Finally, fruit-bearing branches were more efficient at fixing and translocating recent photoassimilates. When these branches were girdled, translocation decreased suggesting that the leaves provide more than enough assimilate for the developing fruit.

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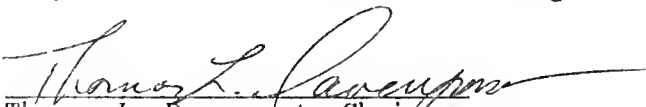
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BIOGRAPHICAL SKETCH

Susan F. Finazzo, daughter of Carlo and Odessie Finazzo, was born in Orlando, FL on May 13, 1957. She attended Caesar Rodney High School in Camden-Wyoming, DE. After graduation, she began studies at the University of Delaware. She graduated in 1979 with a Bachelor of Arts degree in biology and geology. In the fall of that year she began graduate studies at the Pennsylvania State University. She graduated from the Pennsylvania State University in 1982 with Master of Science degree in microbiology.

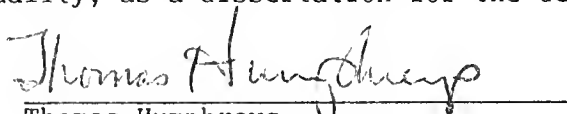
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Thomas L. Davenport, Chairman
Associate Professor of Horticultural Science

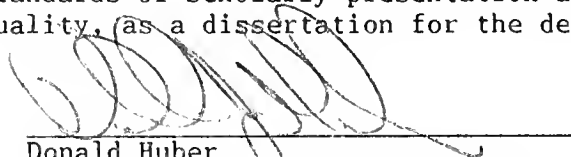
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Karen Koch
Associate Professor of Horticultural Science


I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Thomas Humphreys
Professor of Horticultural Science

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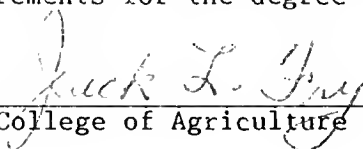

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 1990



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Dean, Graduate School

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